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(54) **NEUROTOXINS EXHIBITING SHORTENED BIOLOGICAL ACTIVITY**

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(57) **ABSTRACT**

The present invention relates to the pharmaceutical field. Specifically, it contemplates a polynucleotide encoding a Neurotoxin polypeptide exhibiting a reduced duration of biological effect in a subject, wherein the polypeptide comprises at least one degradation signal in the light chain as well as vectors and host cells comprising the polynucleotide, polypeptides encoded thereby and antibodies specifically binding to the polypeptides. Moreover, the invention relates to medicaments comprising the polynucleotides and polypeptides as well as specific therapeutic applications thereof. Furthermore, the present invention contemplates methods for the manufacture of the polypeptides and medicaments.

3 Claims, No Drawings

NEUROTOXINS EXHIBITING SHORTENED BIOLOGICAL ACTIVITY

The present invention relates to the pharmaceutical field. Specifically, it contemplates a polynucleotide encoding a Neurotoxin polypeptide exhibiting a reduced duration of the biological effect in a subject, wherein said polypeptide comprises at least one degradation signal in the light chain as well as vectors and host cells comprising the said polynucleotide, polypeptides encoded thereby and antibodies specifically binding to the polypeptides. Moreover, the invention relates to medicaments comprising said polynucleotides and polypeptides as well as specific therapeutic applications thereof. Furthermore, the present invention contemplates methods for the manufacture of the polypeptides and medicaments.

Clostridium botulinum and *Clostridium tetani* produce highly potent neurotoxins, i.e. botulinum toxins (BoNTs) and tetanus toxin (TeNT), respectively. These Clostridial Neurotoxins (CNTs) specifically bind to neuronal cells and disrupt neurotransmitter release. Each toxin is synthesized as an inactive unprocessed approximately 150 kDa single-chain protein. The posttranslational processing involves formation of disulfide bridges, and limited proteolysis (nicking) by the bacterial protease(s). Active Neurotoxin consists of two chains, an N-terminal light chain of approx. 50 kDa and a heavy chain of approx. 100 kDa linked by a disulfide bond. CNTs consist of three domains, i.e. the catalytic light chain, the heavy chain encompassing the translocation domain (N-terminal half) and the receptor binding domain (C-terminal half), see Krieglstein 1990, Eur J Biochem 188, 39; Krieglstein 1991, Eur J Biochem 202, 41; Krieglstein 1994, J Protein Chem 13, 49. The Botulinum Neurotoxins are synthesized as molecular complexes comprising the 150 kDa Neurotoxin protein and associated non-toxic, complexing proteins. The complex sizes differ based on the Clostridial strain and the distinct Neurotoxin serotypes ranging from 300 kDa to 900 kDa. The complexing proteins in these complexes stabilize the Neurotoxin and protect it against degradation, see Chen 1998, Infect Immun 66(6): 2420-2425.

Clostridium botulinum secretes seven antigenically distinct serotypes designated A to G of the botulinum neurotoxin (BoNT). All serotypes together with the related tetanus neurotoxin (TeNT) secreted by *Clostridium tetani*, are Zn^{2+}-endoproteases that block synaptic exocytosis by cleaving SNARE proteins, see Couesnon, 2006, Microbiology, 152, 759. CNTs cause the flaccid muscle paralysis seen in botulism, see Fischer 2007, PNAS 104, 10447.

Despite its toxic effects, Botulinum toxins have been used as therapeutic agents for a large number of diseases or disorders. Botulinum toxin serotype A was approved for human use in the United States in 1989 for the treatment of strabism, blepharospasm, and other disorders. It is commercially available as a Botulinum toxin A protein complex, for example, under the tradename BOTOX (Allergan Inc) or under the tradename DYSPORT (Ipsen Ltd). For therapeutic applications, the complex is injected directly into the muscle to be treated. At physiological pH, the toxin is released from the protein complex and the desired pharmacological effect takes place. An improved, complex-free Neurotoxin A polypeptide preparation is available under the tradename XEOMIN (Merz Pharmaceuticals GmbH). The effect of Botulinum toxin is only temporary, which is the reason why repeated administration of Botulinum toxin may be required to maintain a therapeutic effect.

The Clostridial Neurotoxins weaken voluntary muscle strength and are effective therapeutics for strabism, focal dystonia, including cervical dystonia, and benign essential blepharospasm. They have been further shown to relief hemifacial spasm, and focal spasticity, and, moreover, to be effective in a wide range of other indications, such as gastrointestinal disorders, hyperhidrosis, and cosmetic wrinkle correction, see Jost 2007, Drugs 67, 669.

However, weakening muscle strengths and contraction is also desirable for medical conditions or disease such as wound healing, immobilisation for bone and tendon fracture treatment, post surgery immobilization, specifically in connection with haemorrhoidectomy, introduction of dental implants, or hip joint replacement (endoprothesis), knee arthroplasty, ophthalmological surgery, acne, or irritable bowel disease. The Neurotoxins usually exhibit their biological effect over a time period which is longer than actually needed for efficient treatment of said diseases or conditions. A prolonged muscle paralysis is, however, detrimental or at least less preferable in the therapy of the said medical conditions or diseases. Neurotoxins exhibiting their biological effect only over the desired time period are, however, not yet available.

Accordingly, the technical problem underlying the present invention can be seen as the provision of means and methods for complying with the aforementioned needs. The technical problem is solved by the embodiments characterized in the claims and herein below.

The present invention, therefore, relates to a polynucleotide encoding a Neurotoxin polypeptide exhibiting a reduced duration of the biological effect in a subject, wherein said polypeptide comprises at least one degradation signal in the light chain.

The term "polynucleotide" as used herein refers to single- or double-stranded DNA molecules as well as to RNA molecules. Encompassed by the said term is genomic DNA, cDNA, hnRNA, mRNA as well as all naturally occurring or artificially modified derivatives of such molecular species. The polynucleotide may be in an aspect a linear or circular molecule. Moreover, in addition to the nucleic acid sequences encoding the aforementioned Neurotoxin polypeptide, a polynucleotide of the present invention may comprise additional sequences required for proper transcription and/or translation such as 5' or 3' UTR sequences. The polynucleotide of the present invention encodes a modified Neurotoxin polypeptide derivable from one of the antigenically different serotypes of Botulinum Neurotoxins, i.e. BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G, or Tetanus Neurotoxin (TeNT). In an aspect of the present invention, the said polynucleotide comprises a nucleic acid sequence as shown in SEQ ID NO: 1 (BoNT/A), SEQ ID NO: 3 (BoNT/B), SEQ ID NO: 5 (BoNT/C1), SEQ ID NO: 7 (BoNT/D), SEQ ID NO: 9 (BoNT/E), SEQ ID NO: 11 (BoNT/F), SEQ ID NO: 13 (BoNT/G) or SEQ ID NO: 15 (TeNT). Moreover, encompassed is in an aspect a polynucleotide comprising a nucleic acid sequence encoding an amino acid sequence as shown in any one of SEQ ID NO: 2 (BoNT/A), SEQ ID NO: 4 (BoNT/B), SEQ ID NO: 6 (BoNT/C1), SEQ ID NO: 8 (BoNT/D), SEQ ID NO: 10 (BoNT/E), SEQ ID NO: 12 (BoNT/F), SEQ ID NO: 14 (BoNT/G) or SEQ ID NO: 16 (TeNT). In another aspect, the said polynucleotide is a variant of the aforementioned polynucleotides comprising one or more nucleotide substitutions, deletions and/or additions which in still another aspect may result in an encoded amino acid having one or more amino acid substitutions, deletions and/or additions. Moreover, a variant polynucleotide of the invention shall in another aspect comprise a nucleic acid sequence variant being at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at

least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid sequence as shown in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13 or 15 or a nucleic acid sequence variant which encodes an amino acid sequence being at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence as shown in any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, or 16. The term "identical" as used herein refers to sequence identity characterized by determining the number of identical amino acids between two nucleic acid sequences or amino acid sequences wherein the sequences are aligned so that the highest order match is obtained. It can be calculated using published techniques or methods codified in computer programs such as, for example, BLASTP, BLASTN or FASTA (Altschul 1990, J Mol Biol 215, 403). The percent identity values are, in one aspect, calculated over the entire amino acid sequence. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In this context, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. To carry out the sequence alignments, the program PileUp (Higgins 1989, CABIOS 5, 151) or the programs Gap and BestFit (Needleman 1970, J Mol Biol 48; 443; Smith 1981, Adv Appl Math 2, 482), which are part of the GCG software packet (Genetics Computer Group 1991, 575 Science Drive, Madison, Wis., USA 53711), may be used. The sequence identity values recited above in percent (%) are to be determined, in another aspect of the invention, using the program GAP over the entire sequence region with the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000, which, unless otherwise specified, shall always be used as standard settings for sequence alignments.

In an aspect, each of the aforementioned variant polynucleotides encodes a polypeptide retaining one or more and, in another aspect, all of the biological properties of the respective Neurotoxin polypeptide, i.e. the BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G or Tetanus Neurotoxin (TeNT). Those of skill in the art will appreciate that full biological activity is maintained only after proteolytic activation, even though it is conceivable that the unprocessed precursor can exert some biological functions or be partially active. "Biological properties" as used herein refers to (a) receptor binding, (b) internalization, (c) translocation across the endosomal membrane into the cytosol, and/or (d) endoproteolytic cleavage of proteins involved in synaptic vesicle membrane fusion. In vivo assays for assessing biological activity include the mouse LD50 assay and the ex vivo mouse hemidiaphragm assay as described by Pearce et al. (Pearce 1994, Toxicol Appl Pharmacol 128: 69-77) and Dressler et al. (Dressler 2005, Mov Disord 20:1617-1619, Keller 2006, Neuroscience 139: 629-637). The biological activity is commonly expressed in Mouse Units (MU). As used herein, 1 MU is the amount of neurotoxic component, which kills 50% of a specified mouse population after intraperitoneal injection, i.e. the mouse i.p. LD50. In a further aspect, the variant polynucleotides can encode Neurotoxins having improved or altered biological properties, e.g., they may comprise cleavage sites which are improved for enzyme recognition or may

be improved for receptor binding or any other property specified above. Moreover, encompassed are in an aspect fusion polypeptides further comprising detectable marker peptides or tags. In an aspect, suitable tags are FLAG-tags, Myc-tags or His-tags which also allow for a more efficient purification of the tagged polypeptides. Detectable marker peptides, in an aspect, include fluorescent proteins such as GFP, BFP and the like. In yet a further aspect, the variant polynucleotides shall encode fusion Neurotoxin polypeptides comprising a part of at least two Neurotoxin polypeptides of different serotypes, e.g., a fusion Neurotoxin comprising a heavy chain of BoNT/A and a light chain of BoNT/E.

The Neurotoxin polypeptide encoded by the polynucleotide of the invention further comprises at least one degradation signal in its light chain. In an aspect of the invention, the said light chain of the Neurotoxin polypeptide encoded by the polynucleotide of the invention is obtained by modification from a light chain being encoded by a polynucleotide comprising any one of the aforementioned specific nucleic acid sequences or variants thereof described above. The light chains of the Neurotoxin polypeptides are generated by proteolytic cleavage of a precursor polypeptide (single-chain polypeptide). The light chain is the N-terminal portion of the precursor polypeptide which is obtained as a result of the proteolytic cleavage. The amino acid sequences of the light chains of the Neurotoxin polypeptides referred to above can be deduced, in an aspect, from the cleavage sites indicated in the following table.

TABLE 1

Neurotoxin (Bacterial Strain)	Accession number	Cleavage site	Sequence including cleavage sites (highlighted)
BoNT/A (Hall/62A)	ABD 65472	K428/T429 K448/A449	KLL**C**VRGIITSK**TKSLDKGYNK**ALN....DL**C**IKV (SEQ ID NO: 17)
BoNT/B (Okra)	BAE 48264	K441/A442	IQM**C**KSV**K**APG.....**IC**IDV (SEQ ID NO: 18)
BoNT/C1 (C-6814)	BAA 89713	R444/5445 K449/T450	TKF**C**HKAIDGR**SL....YNK**TL.....DC**R**ELLV (SEQ ID NO: 19)
BoNT/D	BAA 90661	K442/N443 R445/D446	TKV**C**LRLTK.....**NSR**D.....DST**C**IKV (SEQ ID NO: 20)
BoNT/E (Beluga)	CAA 43999	K419/G420 R422/K423	IRF**C**KNIVSV**KG.....IR**K.....SI**C**IEI (SEQ ID NO: 21)
BoNT/F (NCTC10281)	CAA 73972	R435/K436 K439/A440	VKF**C**KSVIPR**KG.....TK**AP.....PRL**C**IRV (SEQ ID NO: 22)

TABLE 1-continued

Neurotoxin (Bacterial Strain)	Accession number	Cleavage site	Sequence including cleavage sites (highlighted)
BoNT/G	CAA 52275		IAM**C**KPVMYKNT.....GKS.....EQ**C**IIV (SEQ ID NO: 23)
TeNT	P 04958	R449 (R455)	IGL**C**KKIIPPTNI**R**ENLYN**R**TASLTDLGGEL**C**IKI (SEQ ID NO: 24)

The term "degradation signal" as used herein refers to modifications of the light chain of the Neurotoxin polypeptide which result in increased degradation of the Neurotoxin polypeptide by endogenous degradation pathways present in an organism to which the Neurotoxin has been applied. In an aspect, the degradation pathway will be a proteasomal degradation pathway or a lysosomal degradation pathway. In another aspect, a degradation pathway may merely result in a partial degradation of the Neurotoxin polypeptide, e.g., by one or more proteolytic cleavage steps. The said degradation signal may be introduced into the light chain (i.e. be located (internally) within the light chain) or linked thereto either N- or C-terminally. The person skilled in the art is well aware of suitable degradation signals and how to introduce or link them to the Neurotoxin polypeptide's light chain. Moreover, the skilled artisan can generate polynucleotides encoding such Neurotoxin polypeptides with the at least one degradation signal by applying recombinant molecular biological techniques or chemical modifications. For example, site directed mutagenesis may be used for introducing the degradation signals referred to below. Alternatively, a nucleic acid sequence for the polynucleotide comprising the coding sequences for the Neurotoxin polypeptide and the envisaged degradation signal may be designed and the entire polynucleotide may subsequently be chemically synthesised.

In an aspect, the said degradation signal is selected from the group consisting of:

a) at least one internally or terminally introduced PEST motif,
b) at least one internally or terminally introduced E3 ligase recognition motif,
c) an N-terminal oligo-lysine residue,
d) an N-terminally linked ubiquitin,
e) a substitution of the N-terminal proline with a basic amino acid,
f) substitutions of surface displayed amino acid residues by lysines, and
g) a substitution of the N-terminal proline with a basic amino acid in combination with substitutions of surface displayed amino acid residues by lysines.

In an aspect, the E3 ligase recognition motif has a consensus sequence as shown in the following table (wherein "X" may represent any of the naturally occurring amino acids):

TABLE 2

E3 ubiquitin Ligase	Recognition motif (consensus)
VBCCul2	ALAPYIP (SEQ ID NO: 25)
MNM2	RFMDYWEGL (SEQ ID NO: 26) FXXXLWXXL (SEQ ID NO: 27)
Smurf2	ELESPPPPYSRYPM (SEQ ID NO: 28)
RN181	KVGFFKR (SEQ ID No: 29)

TABLE 2-continued

E3 ubiquitin Ligase	Recognition motif (consensus)
E3alpha	LLVRGRTLVV (SEQ ID NO 30
SCF	DRHDSGLDSM (SEQ ID NO: 31)
Siah	PXAXVXP (SEQ ID NO: 32)
Itch	PPXYXXM (SEQ ID NO: 33)
Nedd4-2	PPXY (SEQ ID No: 34)

PEST motifs are well known in the art as degradation signals (Rogers 1986, Science 234: 364-368, Rechsteiner 1996, TIBS 21: 267-271, Belizario 2008, Science 9: 210-220). In an aspect, the PEST motif is has a sequence as disclosed in Rechsteiner 1996, TIBS 21: 267-271, Table 1 (hereby incorporated by reference), for any one of the following proteins: GCN4, Iκ Bα, Fos, Ornithine decarboxylase, Cactus, CLN2, CLN 3 or NIMA.

The modified Neurotoxin polypeptide encoded by the polynucleotide of the present invention will exhibit a reduced duration of the biological effect in a subject upon administration in comparison to an unmodified Neurotoxin polypeptide. In an aspect, the said biological effect observed in the subject causes muscle paralysis, i.e. a (reversible) inactivation of the muscle's capability to contract. In an aspect, the effects can be tested by the so-called mouse running assay (Keller 2006, Neuroscience 139: 629-637). The biological effects can be determined by the person skilled in the art without further ado. A reduced duration of the biological effect, in an aspect, refers to a statistically significant reduced duration. Whether the duration of an effect is statistically significant reduced can be determined by those skilled in the art by applying standard statistical tests, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99%. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the probability envisaged by the present invention allows that the diagnosis will be correct for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population. In an aspect, the said reduced duration persist less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 30% or less than 20% of the normal duration, i.e. the duration observed for an unmodified Neurotoxin polypeptide. In an aspect, normal duration persists for approximately 4 month in the case of BoNT/A, 2 months in the case of BoNT/B, approximately 3 to 4 months in the case of BoNT/C or approximately 4 weeks in the case of BoNT/E (Foran, J Biol. Chem. 278(2): 1363-1371, Eleopra 1998, Neurosci Lett. 13, 256(3): 135-138, Eleopra 1997, Neurosci Lett.

14,224(2): 91-94, Sloop 1997, Neurology 49(1): 189-194, Washbourne 1998, J Physiol Paris 92(2): 135-139). It is to be understood that the duration of the effect depends on individual influences in a subject such as genetic background, age, life style etc. Therefore, an approximate duration as meant herein refers to a duration as indicated above for the respective Neurotoxin polypeptides (e.g., 4 months for BoNT/A or 4 weeks for BoNT/E) with a standard deviation of 25% or less, 20% or less, 15% or less, 10% or less or 5% or less.

Advantageously, it has been found in accordance with the present invention that a Neurotoxin polypeptide can be modified to exhibit a shortened biological effect in a subject upon administration. In principle, this can be achieved by introducing or linking a degradation signal to the light chain of the said Neurotoxin polypeptide since it was found that the persistence of the light chain correlates with the duration of the biological effect. The shortened duration of the biological effect elicited by Neurotoxin polypeptides is beneficial for various medical applications which require an inactivation of nervous actions, e.g., muscle paralysis in order to facilitate wound healing.

The present invention contemplates a vector comprising the polynucleotide of the present invention.

The term "vector", preferably, encompasses phage, plasmid, viral or retroviral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site-directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homologous or heterologous recombination as described in detail below. The vector encompassing the polynucleotides of the present invention, in an aspect, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. For example, a plasmid vector can be introduced in a precipitate such as a calcium phosphate precipitate or rubidium chloride precipitate, or in a complex with a charged lipid or in carbon-based clusters, such as fullerens. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host/cells. Moreover, in an aspect of the invention, the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic host cells or isolated fractions thereof in the said vector. Expression of the polynucleotide comprises transcription of the polynucleotide into a translatable mRNA. Regulatory elements ensuring expression in host cells are well known in the art. In an aspect, they comprise regulatory sequences ensuring initiation of transcription and/or poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the lac-, trp- or tac-promoter in *E. coli*, and examples for regulatory elements permitting expression in eukaryotic host cells are the AOX1- or the GAL1-promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Moreover, inducible expression control sequences may be used in an expression vector encompassed by the present invention. Such inducible vectors may comprise tet or lac operator sequences or sequences inducible by heat shock or other environmental factors. Suitable expression control sequences are well known in the art. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pBluescript (Stratagene), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen) or pSPORT1 (Invitrogen). Preferably, said vector is an expression vector and a gene transfer or targeting vector. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector of the invention into targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors; see, for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1994).

Moreover, the present invention pertains to a host cell comprising the polynucleotide or the vector of the present invention.

The term "host cell" as used herein encompasses prokaryotic and eukaryotic host cells. In an aspect the host cell is a bacterial cell and, in another aspect, a Firmicutes bacterial cell. In one aspect, the said bacterial host cell is an *E. coli* host cell. In another aspect, it is a *Clostridium* host cell. In a further aspect, the said *Clostridium* host cell is a *Clostridium botulinum* host cell, in even a further aspect, a cell of one of the aforementioned seven different serotypes of *Clostridium botulinum*. In yet another aspect, the bacterial host cell is a *Clostridium tetani* host cell. In a further aspect, the host cell is a *Bacillus* host cell and in a particular aspect a *Bacillus megaterium* host cell. A eukaryotic host cell, in an aspect, is a cell of an animal cell line suitable for production of toxic proteins or a fungal host cell such as a yeast host cell.

Also encompassed by the present invention is a polypeptide encoded by the polynucleotide of the invention.

The term "polypeptide" as used herein encompasses isolated or essentially purified polypeptides being essentially free of other polypeptides including the complexing proteins (HA70, HA17, HA33, or NTNH (NBP) of the host cell or polypeptide preparations comprising other proteins in addition. Moreover, the term includes chemically modified polypeptides. Such modifications may be artificial modifications or naturally occurring modifications. As referred to above, the polypeptide of the present invention shall have the biological properties of the Neurotoxin polypeptides referred to above. Moreover, it shall exhibit shortened duration of the biological effect in a subject upon administration. The polypeptide of the invention, in an aspect, can be manufactured by a method of manufacturing a polypeptide as described elsewhere herein in more detail. In an aspect of the invention, a polypeptide preparation is also envisaged which comprises a complex of the Neurotoxin polypeptide and its complexing proteins.

Moreover, the present invention relates to an antibody which specifically binds to the polypeptide of the present invention.

Antibodies against the polypeptide of the invention can be prepared by well known methods using a purified polypeptide according to the invention or a suitable fragment derived

therefrom as an antigen. A fragment which is suitable as an antigen may be identified by antigenicity determining algorithms well known in the art. Such fragments may be obtained either from the polypeptide of the invention by proteolytic digestion or may be a synthetic peptide. In an aspect, the antibody of the present invention is a monoclonal antibody, a polyclonal antibody, a single chain antibody, a human or humanized antibody or primatized, chimerized or fragment thereof. Also comprised as antibodies by the present invention is a bispecific antibody, a synthetic antibody, an antibody fragment, such as a Fab, Fv or scFv fragment etc., or a chemically modified derivative of any of these. The antibody of the present invention shall specifically bind (i.e. does not cross react with other polypeptides or peptides) to the polypeptide of the invention. Specifically, the antibody shall also not cross react with the unmodified Neurotoxin polypeptide. Specific binding can be tested by various well known techniques. Antibodies or fragments thereof can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. Monoclonal antibodies can be prepared by the techniques originally described bei Köhler et al. (Köhler 1975, Nature 256 (1975), 495) or Galfré. (Galfré 1981, Meth. Enzymol. 73 (1981)) which comprise the fusion of mouse myeloma cells to spleen cells derived from mammals which have been immunized by the antigen, i.e. the polypeptide of the invention or a immunogenic fragment thereof. The antibodies can be used, for example, for the immunoprecipitation and immunolocalization of the polypeptides of the invention as well as for the monitoring of the presence of said polypeptides, for example, in recombinant organisms, and for the identification of compounds interacting with the proteins according to the invention. For example, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to an epitope of the protein of the invention (Schier 1996, Human Antibodies Hybridomas 7, 97-105; Malmborg 1995, J. Immunol. Methods 183, 7-13).

The polynucleotide or polypeptide of the invention can be used as a medicament, in general.

The term "medicament" as used herein refers, in one aspect, to a pharmaceutical composition containing the biologically active Neurotoxin polypeptide or a polynucleotide encoding it as pharmaceutical active compound. The said medicament may be used for human or animal therapy of various diseases or disorders in a therapeutically effective dose. The medicament can be formulated by various techniques dependent on the desired application purposes. Different aspects of a medicament according to the present invention are specified herein below.

In an aspect, the medicament comprises the biologically active Neurotoxin polypeptide of the present invention one or more pharmaceutically acceptable carrier as a pharmaceutical composition. The pharmaceutically acceptable carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and being not deleterious to the recipient thereof. The pharmaceutical carrier employed may include a solid, a gel, or a liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are glycerol, phosphate buffered saline solution, water, emulsions, various types of wetting agents, and the like. Suitable carriers comprise those mentioned above and others well known in the art, see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa. It will be understood that a carrier might also be a virus or retrovirus suitable for gene therapy, in particular, if the active ingredient of the medicament is the polynucleotide of the invention.

The medicament, in an aspect, will be dissolved in a diluent prior to administration. The diluent is also selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water or physiological saline. In addition, the pharmaceutical composition or formulation may also include other carriers or non-toxic, non-therapeutic, non-immunogenic stabilizers and the like. Thus, the Neurotoxin polypeptide of the invention can be present, in an aspect, in liquid or lyophilized form. In an aspect, it can be present together with glycerol, protein stabilizers (HSA) or non-protein stabilizers such as polyviylpyrolidon (PVP), hyaluronic acid or free amino acids. In an aspect, suitable non-proteinaceous stabilizers are disclosed in WO 2005/007185 or WO 2006/020208.

In another aspect, the medicament will be provided as a solution comprising the Neurotoxin polypeptide. Moreover, the solution can comprise carriers or stabilizers referred to above as well. A stable liquid formulation of the Neurotoxin polypeptide can be provided, in an aspect, as disclosed by U.S. Pat. No. 7,211,261.

The pharmaceutical composition is, in one aspect, administered topically. Conventionally the medicament will be administered intra-muscular or subcutaneous (near glands) depending on the desired medical indication. However, depending on the nature and the mode of action of a compound the pharmaceutical composition may be administered by other routes as well.

A therapeutically effective dose refers to an amount of the Neurotoxin polypeptide or the polynucleotide of the invention which prevents, ameliorates or treats the symptoms accompanying a condition or disease referred to in this specification. Therapeutic efficacy and toxicity of the compound can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. The medicament of the present invention will comprise, in an aspect, dosage recommendations in the prescribers or users instructions in order to anticipate dosage adjustments depending on the individual recipient.

The medicament referred to herein are developed to be administered at least once in order to treat or ameliorate or prevent a disease or condition recited in this specification. However, the said medicament may be administered more than one time.

The medicament according to the present invention may in a further aspect of the invention comprise drugs in addition to the biologically active Neurotoxin polypeptide which are added to the pharmaceutical composition during its formulation.

Moreover, the present invention pertains to the use of the polynucleotide or the polypeptide of the present invention for the preparation of a medicament for the treatment of wound healing, immobilization for bone and tendon fracture treatment, post surgery immobilization, specifically in connection with haemorrhoidectomy, introduction of dental implants, or hip joint replacement (endoprothesis), knee arthroplasty, ophthalmological surgery, acne, or irritable bowel disease.

The symptoms associated with the aforementioned medical conditions or diseases are well known to the person skilled in the art and are described in standard text books of medicine such as Stedman or Pschyrembl.

Moreover, the present invention also relates to the use of the polynucleotide or the polypeptide of the present invention for the preparation of a diagnostic medicament for determining whether a subject is susceptible for a Neurotoxin therapy.

The diagnostic medicament referred to above is a Neurotoxin polypeptide medicament as referred to above. However, the medicament is to be applied for a time and at a dosage regimen allowing merely the determination of whether a subject responds to the Neurotoxin polypeptide at all or the determination of a suitable dosage regimen. Since the above Neurotoxin polypeptide—although having therapeutic potential as well—is pivotally used for a diagnostic purpose rather than for treating or amelioration in this aspect, the medicament comprising it is termed "diagnostic medicament". Thus, such a time-restricted pre-screen with the modified Neurotoxin polypeptides of the present invention will assist in selecting subjects susceptible for a therapy using an unmodified Neurotoxin as well as in determining a suitable dosage. Potential side effects of a therapy based on an unmodified Neurotoxin which would normally persist over a longer time can be reduced due to the reduced duration of the biological effect elicited by the modified Neurotoxin polypeptide of the invention.

The present invention encompasses a method for the manufacture of a Neurotoxin polypeptide encoded by the polynucleotide of the invention comprising the steps of:

a) cultivating the host cell of the invention under conditions which allow for the expression of the Neurotoxin polypeptide encoded by the polynucleotide of the invention, and

b) obtaining the Neurotoxin polypeptide encoded by the polynucleotide of the invention from the host cell culture of a).

The polypeptide may be obtained from the culture, in an aspect, by all conventional purification techniques including affinity chromatography, size exclusion chromatography, high pressure liquid chromatography (HPLC) and precipitation techniques including antibody precipitation. Moreover, in an aspect the Neurotoxin polypeptide obtained by the method of the invention may be free of complexing proteins. In another aspect, the Neurotoxin polypeptide may be obtained as a complex comprising in addition to the Neurotoxin polypeptide complexing proteins. Moreover, obtaining as used herein, in an aspect, includes activation of the Neurotoxin polypeptide. This can be achieved by proteolytic cleavage of the (single-chain) Neurotoxin polypeptide precursor either intracellular by an endogenous or exogenous (e.g., recombinant expressed) protease or outside the cell by contacting the Neurotoxin polypeptide, e.g., prior, during or after the aforementioned purification, with the protease under conditions allowing for cleavage.

Furthermore, a method for the manufacture of a medicament is contemplated in accordance with the present invention, said method comprising the steps of the aforementioned method of the invention and the further step of formulating the Neurotoxin polypeptide encoded by the polynucleotide of the invention as a medicament.

It will be understood that such a method for the manufacture of a medicament is carried out according to the GMP standards for medicaments in order to ensure quality, pharmaceutical safety, and efficacy of the medicament. Suitable formulations of the medicament are described elsewhere in this specification. The person skilled in the art is, however, well aware of how such formulations can be made.

The invention also encompasses a method for the manufacture of a cosmetic composition comprising the steps of the method of the invention and the further step of formulating the Neurotoxin polypeptide as a cosmetic composition.

"Cosmetic composition" as used herein can be formulated as described for a pharmaceutical composition above. For a cosmetic composition, likewise, it is envisaged that the compound of the present invention is in an aspect used in substantially pure form. Impurities, however, may be less critical than for a medicament. Cosmetic compositions are, in a further aspect, to be applied intramuscular. In an even further aspect of the invention, cosmetic compositions comprising the Neurotoxin can be formulated as an anti-wrinkle agent.

The present invention also pertains to such a cosmetic composition and to the use of the polynucleotide or the polypeptide of the present invention for the preparation of a cosmetic composition to be used as an anti-wrinkle agent.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

SEQUENCE LISTING

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<212> TYPE: PRT
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Asp Asn Glu Lys Asp Asn Tyr Leu Lys Gly Val Thr Lys Leu Phe Glu
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Arg Gly Ile Pro Phe Trp Gly Gly Ser Thr Ile Asp Thr Glu Leu Lys
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Val Ile Asp Thr Asn Cys Ile Asn Val Ile Gln Pro Asp Gly Ser Tyr
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Glu Val Ser Phe Glu Glu Leu Arg Thr Phe Gly Gly His Asp Ala Lys
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Lys Phe Lys Asp Ile Ala Ser Thr Leu Asn Lys Ala Lys Ser Ile Val
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Tyr Leu Leu Ser Glu Asp Thr Ser Gly Lys Phe Ser Val Asp Lys Leu
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Lys Phe Asp Lys Leu Tyr Lys Met Leu Thr Glu Ile Tyr Thr Glu Asp
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Asn Phe Val Lys Phe Phe Lys Val Leu Asn Arg Lys Thr Tyr Leu Asn
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Phe Asp Lys Ala Val Phe Lys Ile Asn Ile Val Pro Lys Val Asn Tyr
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Leu Asp Lys Tyr Thr Met Phe His Tyr Leu Arg Ala Gln Glu Phe Glu
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His Gly Lys Ser Arg Ile Ala Leu Thr Asn Ser Val Asn Glu Ala Leu
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Leu Asn Ile Gly Asn Met Leu Tyr Lys Asp Asp Phe Val Gly Ala Leu
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Ile Phe Ser Gly Ala Val Ile Leu Leu Glu Phe Ile Pro Glu Ile Ala
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Ile Pro Val Leu Gly Thr Phe Ala Leu Val Ser Tyr Ile Ala Asn Lys
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Val Leu Thr Val Gln Thr Ile Asp Asn Ala Leu Ser Lys Arg Asn Glu
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Lys Trp Asp Glu Val Tyr Lys Tyr Ile Val Thr Asn Trp Leu Ala Lys
705                 710                 715                 720

Val Asn Thr Gln Ile Asp Leu Ile Arg Lys Lys Met Lys Glu Ala Leu
                725                 730                 735

Glu Asn Gln Ala Glu Ala Thr Lys Ala Ile Ile Asn Tyr Gln Tyr Asn
            740                 745                 750

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Asp Ala Leu Leu Lys Tyr Ile Tyr Asp Asn Arg Gly Thr Leu Ile Gly
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Ile Pro Phe Gln Leu Ser Lys Tyr Val Asp Asn Gln Arg Leu Leu Ser
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Thr Phe Thr Glu Tyr Ile Lys Asn Ile Ile Asn Thr Ser Ile Leu Asn
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Leu Arg Tyr Glu Ser Asn His Leu Ile Asp Leu Ser Arg Tyr Ala Ser
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Lys Ile Asn Ile Gly Ser Lys Val Asn Phe Asp Pro Ile Asp Lys Asn
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Gln Ile Gln Leu Phe Asn Leu Glu Ser Ser Lys Ile Glu Val Ile Leu
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Lys Asn Ala Ile Val Tyr Asn Ser Met Tyr Glu Asn Phe Ser Thr Ser
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Phe Trp Ile Arg Ile Pro Lys Tyr Phe Asn Ser Ile Ser Leu Asn Asn
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Glu Tyr Thr Ile Ile Asn Cys Met Glu Asn Asn Ser Gly Trp Lys Val
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Ser Leu Asn Tyr Gly Glu Ile Ile Trp Thr Leu Gln Asp Thr Gln Glu
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gatagactta acaaggtttt agtttgcata tcagatccta acattaatat taatatatat 960

aaaaataaat ttaaagataa atataaattc gttgaagatt ctgagggaaa atatagtata 1020

gatgtagaaa gttttgataa attatataaa agcttaatgt ttggttttac agaaactaat 1080

atagcagaaa attataaaat aaaaactaga gcttcttatt ttagtgattc cttaccacca 1140

gtaaaaataa aaaatttatt agataatgaa atctatacta tagaggaagg gtttaatata 1200

tctgataaag atatggaaaa agaatataga ggtcagaata aagctataaa taaacaagct 1260

tatgaagaaa ttagcaagga gcatttggct gtatataaga tacaaatgtg taaaagtgtt 1320

aaagctccag gaatatgtat tgatgttgat aatgaagatt tgttctttat agctgataaa 1380

aatagttttt cagatgattt atctaaaaac gaaagaatag aatataatac acagagtaat 1440

tatatagaaa atgacttccc tataaatgaa ttaattttag atactgattt aataagtaaa 1500

atagaattac caagtgaaaa tacagaatca cttactgatt ttaatgtaga tgttccagta 1560

tatgaaaaac aacccgctat aaaaaaaatt tttacagatg aaaataccat ctttcaatat 1620

ttatactctc agacatttcc tctagatata agagatataa gtttaacatc ttcatttgat 1680

gatgcattat tattttctaa caaagtttat tcatttttt ctatggatta tattaaaact 1740

gctaataaag tggtagaagc aggattattt gcaggttggg tgaaacagat agtaaatgat 1800

tttgtaatcg aagctaataa aagcaatact atggataaaa ttgcagatat atctctaatt 1860

gttccttata taggattagc tttaaatgta ggaaatgaaa cagctaaagg aaattttgaa 1920

aatgcttttg agattgcagg agccagtatt ctactagaat ttataccaga acttttaata 1980

cctgtagttg gagccttttt attagaatca tatattgaca ataaaaataa aattattaaa 2040

acaatagata atgctttaac taaaagaaat gaaaaatgga gtgatatgta cggattaata 2100

gtagcgcaat ggctctcaac agttaatact caattttata caataaaaga gggaatgtat 2160

aaggctttaa attatcaagc acaagcattg gaagaaataa taaaatacag atataatata 2220

tattctgaaa aagaaaagtc aaatattaac atcgatttta atgatataaa ttctaaactt 2280

aatgagggta ttaaccaagc tatagataat ataaataatt ttataaatgg atgttctgta 2340

tcatatttaa tgaaaaaaat gattccatta gctgtagaaa aattactaga ctttgataat 2400

actctcaaaa aaaatttgtt aaattatata gatgaaaata aattatattt gattggaagt 2460

gcagaatatg aaaaatcaaa agtaaataaa tacttgaaaa ccattatgcc gtttgatctt 2520

tcaatatata ccaatgatac aatactaata gaaatgttta ataaatataa tagcgaaatt 2580

ttaaataata ttatcttaaa tttaagatat aaggataata atttaataga tttatcagga 2640

tatggggcaa aggtagaggt atatgatgga gtcgagctta atgataaaaa tcaatttaaa 2700

ttaactagtt cagcaaatag taagattaga gtgactcaaa atcagaatat catatttaat 2760

agtgtgttcc ttgattttag cgttagcttt tggataagaa tacctaaata taagaatgat 2820

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ggtatacaaa attatattca taatgaatat acaataatta attgtatgaa aaataattcg 2880

ggctggaaaa tatctattag gggtaatagg ataatatgga ctttaattga tataaatgga 2940

aaaaccaaat cggtattttt tgaatataac ataagagaag atatatcaga gtatataaat 3000

agatggtttt ttgtaactat tactaataat ttgaataacg ctaaaattta tattaatggt 3060

aagctagaat caaatacaga tattaaagat ataagagaag ttattgctaa tggtgaaata 3120

atatttaaat tagatggtga tatagataga acacaattta tttggatgaa atatttcagt 3180

atttttaata cggaattaag tcaatcaaat attgaagaaa gatataaaat tcaatcatat 3240

agcgaatatt taaaagattt ttggggaaat cctttaatgt acaataaaga atattatatg 3300

tttaatgcgg ggaataaaaa ttcatatatt aaactaaaga aagattcacc tgtaggtgaa 3360

attttaacac gtagcaaata taatcaaaat tctaaatata taaattatag agatttatat 3420

attggagaaa aatttattat aagaagaaag tcaaattctc aatctataaa tgatgatata 3480

gttagaaaag aagattatat atatctagat ttttttaatt taaatcaaga gtggagagta 3540

tatacctata aatattttaa gaaagaggaa gaaaaattgt ttttagctcc tataagtgat 3600

tctgatgagt tttacaatac tatacaaata aaagaatatg atgaacagcc aacatatagt 3660

tgtcagttgc tttttaaaaa agatgaagaa agtactgatg agataggatt gattggtatt 3720

catcgtttct acgaatctgg aattgtattt gaagagtata aagattattt ttgtataagt 3780

aaatggtact taaaagaggt aaaaaggaaa ccatataatt taaaattggg atgtaattgg 3840

cagtttattc ctaaagatga agggtggact gaataa 3876

<210> SEQ ID NO 4
<211> LENGTH: 1291
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 4

Met Pro Val Thr Ile Asn Asn Phe Asn Tyr Asn Asp Pro Ile Asp Asn
1 5 10 15

Asn Asn Ile Ile Met Met Glu Pro Pro Phe Ala Arg Gly Thr Gly Arg
20 25 30

Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Ile Pro Glu
35 40 45

Arg Tyr Thr Phe Gly Tyr Lys Pro Glu Asp Phe Asn Lys Ser Ser Gly
50 55 60

Ile Phe Asn Arg Asp Val Cys Glu Tyr Tyr Asp Pro Asp Tyr Leu Asn
65 70 75 80

Thr Asn Asp Lys Lys Asn Ile Phe Leu Gln Thr Met Ile Lys Leu Phe
85 90 95

Asn Arg Ile Lys Ser Lys Pro Leu Gly Glu Lys Leu Leu Glu Met Ile
100 105 110

Ile Asn Gly Ile Pro Tyr Leu Gly Asp Arg Arg Val Pro Leu Glu Glu
115 120 125

Phe Asn Thr Asn Ile Ala Ser Val Thr Val Asn Lys Leu Ile Ser Asn
130 135 140

Pro Gly Glu Val Glu Arg Lys Lys Gly Ile Phe Ala Asn Leu Ile Ile
145 150 155 160

Phe Gly Pro Gly Pro Val Leu Asn Glu Asn Glu Thr Ile Asp Ile Gly
165 170 175

Ile Gln Asn His Phe Ala Ser Arg Glu Gly Phe Gly Gly Ile Met Gln
180 185 190

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Met Lys Phe Cys Pro Glu Tyr Val Ser Val Phe Asn Asn Val Gln Glu
        195                 200                 205

Asn Lys Gly Ala Ser Ile Phe Asn Arg Arg Gly Tyr Phe Ser Asp Pro
    210                 215                 220

Ala Leu Ile Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225                 230                 235                 240

Gly Ile Lys Val Asp Asp Leu Pro Ile Val Pro Asn Glu Lys Lys Phe
                245                 250                 255

Phe Met Gln Ser Thr Asp Ala Ile Gln Ala Glu Glu Leu Tyr Thr Phe
            260                 265                 270

Gly Gly Gln Asp Pro Ser Ile Ile Thr Pro Ser Thr Asp Lys Ser Ile
        275                 280                 285

Tyr Asp Lys Val Leu Gln Asn Phe Arg Gly Ile Val Asp Arg Leu Asn
    290                 295                 300

Lys Val Leu Val Cys Ile Ser Asp Pro Asn Ile Asn Ile Asn Ile Tyr
305                 310                 315                 320

Lys Asn Lys Phe Lys Asp Lys Tyr Lys Phe Val Glu Asp Ser Glu Gly
                325                 330                 335

Lys Tyr Ser Ile Asp Val Glu Ser Phe Asp Lys Leu Tyr Lys Ser Leu
            340                 345                 350

Met Phe Gly Phe Thr Glu Thr Asn Ile Ala Glu Asn Tyr Lys Ile Lys
        355                 360                 365

Thr Arg Ala Ser Tyr Phe Ser Asp Ser Leu Pro Pro Val Lys Ile Lys
    370                 375                 380

Asn Leu Leu Asp Asn Glu Ile Tyr Thr Ile Glu Glu Gly Phe Asn Ile
385                 390                 395                 400

Ser Asp Lys Asp Met Glu Lys Glu Tyr Arg Gly Gln Asn Lys Ala Ile
                405                 410                 415

Asn Lys Gln Ala Tyr Glu Glu Ile Ser Lys Glu His Leu Ala Val Tyr
            420                 425                 430

Lys Ile Gln Met Cys Lys Ser Val Lys Ala Pro Gly Ile Cys Ile Asp
        435                 440                 445

Val Asp Asn Glu Asp Leu Phe Phe Ile Ala Asp Lys Asn Ser Phe Ser
    450                 455                 460

Asp Asp Leu Ser Lys Asn Glu Arg Ile Glu Tyr Asn Thr Gln Ser Asn
465                 470                 475                 480

Tyr Ile Glu Asn Asp Phe Pro Ile Asn Glu Leu Ile Leu Asp Thr Asp
                485                 490                 495

Leu Ile Ser Lys Ile Glu Leu Pro Ser Glu Asn Thr Glu Ser Leu Thr
            500                 505                 510

Asp Phe Asn Val Asp Val Pro Val Tyr Glu Lys Gln Pro Ala Ile Lys
        515                 520                 525

Lys Ile Phe Thr Asp Glu Asn Thr Ile Phe Gln Tyr Leu Tyr Ser Gln
    530                 535                 540

Thr Phe Pro Leu Asp Ile Arg Asp Ile Ser Leu Thr Ser Ser Phe Asp
545                 550                 555                 560

Asp Ala Leu Leu Phe Ser Asn Lys Val Tyr Ser Phe Phe Ser Met Asp
                565                 570                 575

Tyr Ile Lys Thr Ala Asn Lys Val Val Glu Ala Gly Leu Phe Ala Gly
            580                 585                 590

Trp Val Lys Gln Ile Val Asn Asp Phe Val Ile Glu Ala Asn Lys Ser
        595                 600                 605
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Asn Thr Met Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr Ile
    610                 615                 620

Gly Leu Ala Leu Asn Val Gly Asn Glu Thr Ala Lys Gly Asn Phe Glu
625                 630                 635                 640

Asn Ala Phe Glu Ile Ala Gly Ala Ser Ile Leu Leu Glu Phe Ile Pro
                645                 650                 655

Glu Leu Leu Ile Pro Val Val Gly Ala Phe Leu Leu Glu Ser Tyr Ile
            660                 665                 670

Asp Asn Lys Asn Lys Ile Ile Lys Thr Ile Asp Asn Ala Leu Thr Lys
        675                 680                 685

Arg Asn Glu Lys Trp Ser Asp Met Tyr Gly Leu Ile Val Ala Gln Trp
    690                 695                 700

Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile Lys Glu Gly Met Tyr
705                 710                 715                 720

Lys Ala Leu Asn Tyr Gln Ala Gln Ala Leu Glu Glu Ile Ile Lys Tyr
                725                 730                 735

Arg Tyr Asn Ile Tyr Ser Glu Lys Glu Lys Ser Asn Ile Asn Ile Asp
            740                 745                 750

Phe Asn Asp Ile Asn Ser Lys Leu Asn Glu Gly Ile Asn Gln Ala Ile
        755                 760                 765

Asp Asn Ile Asn Asn Phe Ile Asn Gly Cys Ser Val Ser Tyr Leu Met
    770                 775                 780

Lys Lys Met Ile Pro Leu Ala Val Glu Lys Leu Leu Asp Phe Asp Asn
785                 790                 795                 800

Thr Leu Lys Lys Asn Leu Leu Asn Tyr Ile Asp Glu Asn Lys Leu Tyr
                805                 810                 815

Leu Ile Gly Ser Ala Glu Tyr Glu Lys Ser Lys Val Asn Lys Tyr Leu
            820                 825                 830

Lys Thr Ile Met Pro Phe Asp Leu Ser Ile Tyr Thr Asn Asp Thr Ile
        835                 840                 845

Leu Ile Glu Met Phe Asn Lys Tyr Asn Ser Glu Ile Leu Asn Asn Ile
    850                 855                 860

Ile Leu Asn Leu Arg Tyr Lys Asp Asn Asn Leu Ile Asp Leu Ser Gly
865                 870                 875                 880

Tyr Gly Ala Lys Val Glu Val Tyr Asp Gly Val Glu Leu Asn Asp Lys
                885                 890                 895

Asn Gln Phe Lys Leu Thr Ser Ser Ala Asn Ser Lys Ile Arg Val Thr
            900                 905                 910

Gln Asn Gln Asn Ile Ile Phe Asn Ser Val Phe Leu Asp Phe Ser Val
        915                 920                 925

Ser Phe Trp Ile Arg Ile Pro Lys Tyr Lys Asn Asp Gly Ile Gln Asn
    930                 935                 940

Tyr Ile His Asn Glu Tyr Thr Ile Ile Asn Cys Met Lys Asn Asn Ser
945                 950                 955                 960

Gly Trp Lys Ile Ser Ile Arg Gly Asn Arg Ile Ile Trp Thr Leu Ile
                965                 970                 975

Asp Ile Asn Gly Lys Thr Lys Ser Val Phe Phe Glu Tyr Asn Ile Arg
            980                 985                 990

Glu Asp Ile Ser Glu Tyr Ile Asn  Arg Trp Phe Phe Val  Thr Ile Thr
        995                 1000                1005

Asn Asn  Leu Asn Asn Ala Lys  Ile Tyr Ile Asn Gly  Lys Leu Glu
    1010                1015                1020

Ser Asn  Thr Asp Ile Lys Asp  Ile Arg Glu Val Ile  Ala Asn Gly
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    1025                1030                1035

Glu Ile  Ile Phe Lys Leu Asp  Gly Asp Ile Asp Arg  Thr Gln Phe
    1040                1045                1050

Ile Trp  Met Lys Tyr Phe Ser  Ile Phe Asn Thr Glu  Leu Ser Gln
    1055                1060                1065

Ser Asn  Ile Glu Glu Arg Tyr  Lys Ile Gln Ser Tyr  Ser Glu Tyr
    1070                1075                1080

Leu Lys  Asp Phe Trp Gly Asn  Pro Leu Met Tyr Asn  Lys Glu Tyr
    1085                1090                1095

Tyr Met  Phe Asn Ala Gly Asn  Lys Asn Ser Tyr Ile  Lys Leu Lys
    1100                1105                1110

Lys Asp  Ser Pro Val Gly Glu  Ile Leu Thr Arg Ser  Lys Tyr Asn
    1115                1120                1125

Gln Asn  Ser Lys Tyr Ile Asn  Tyr Arg Asp Leu Tyr  Ile Gly Glu
    1130                1135                1140

Lys Phe  Ile Ile Arg Arg Lys  Ser Asn Ser Gln Ser  Ile Asn Asp
    1145                1150                1155

Asp Ile  Val Arg Lys Glu Asp  Tyr Ile Tyr Leu Asp  Phe Phe Asn
    1160                1165                1170

Leu Asn  Gln Glu Trp Arg Val  Tyr Thr Tyr Lys Tyr  Phe Lys Lys
    1175                1180                1185

Glu Glu  Glu Lys Leu Phe Leu  Ala Pro Ile Ser Asp  Ser Asp Glu
    1190                1195                1200

Phe Tyr  Asn Thr Ile Gln Ile  Lys Glu Tyr Asp Glu  Gln Pro Thr
    1205                1210                1215

Tyr Ser  Cys Gln Leu Leu Phe  Lys Lys Asp Glu Glu  Ser Thr Asp
    1220                1225                1230

Glu Ile  Gly Leu Ile Gly Ile  His Arg Phe Tyr Glu  Ser Gly Ile
    1235                1240                1245

Val Phe  Glu Glu Tyr Lys Asp  Tyr Phe Cys Ile Ser  Lys Trp Tyr
    1250                1255                1260

Leu Lys  Glu Val Lys Arg Lys  Pro Tyr Asn Leu Lys  Leu Gly Cys
    1265                1270                1275

Asn Trp  Gln Phe Ile Pro Lys  Asp Glu Gly Trp Thr  Glu
    1280                1285                1290
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<210> SEQ ID NO 5
<211> LENGTH: 3843
<212> TYPE: DNA
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 5

atgccaataa caattaacaa ctttaattat tcagatcctg ttgataataa aaatatttta     60

tatttagata ctcatttaaa tacattagct aatgagcctg aaaaagcctt tcgcattata    120

gggaatatat gggtaatacc cgatagattt tcaagagatt ctaatccaaa tttaaataaa    180

cctcctcgag ttacaagccc taaaagtggt tattatgatc ctaattattt gagtactgat    240

tctgaaaaag atacattttt aaaagaaatt ataaagttat ttaaaagaat taactctaga    300

gaaataggag aagaattaat atatagactt gcaacagaca tacccttcc tgggaataac    360

aatactccaa ttaatacttt tgattttgat gtagatttta acagtgttga tgttaaaact    420

agacaaggta acaactgggt taaaactggt agtataaatc ctagtgttat aataactgga    480

cctagagaaa acattataga cccagaaact tctacgttta aattaactaa caatactttt    540
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gcggcacaag aaggatttgg tgctttatca ataatttcaa tatcacctag atttatgcta 600

acatatagta atgcaactaa taatgtagga gagggtagat tttctaagtc tgaattttgc 660

atggatccaa tactaatttt aatgcatgaa cttaatcatg caatgcataa tttatatgga 720

atagctatac caaatgatca aagaatttca tctgtaacta gtaatatttt ttattctcaa 780

tataaggtga aattagagta tgcagaaata tatgcatttg gaggtccaac tatagacctt 840

attcctaaaa gtgcaaggaa atattttgag gaaaaggcat tggattatta tagatccata 900

gctaaaagac ttaatagtat aactactgca aatccttcaa gctttaataa atatatagga 960

gaatataaac agaaacttat tagaaagtat agattcgtag tagaatcttc aggtgaagtt 1020

gcagtagatc gtaataagtt tgctgagtta tataaagaac ttacacaaat atttacagaa 1080

tttaactacg ctaaaatata taatgtacaa aataggaaaa tatatctttc aaatgtatat 1140

actccggtta cggcaaatat attagacgat aatgtttatg atatacaaaa tggatttaac 1200

atacctaaaa gtaatttaaa tgtactattt atgggtcaaa atttatctcg aaatccagca 1260

ttaagaaaag tcaatcctga aaatatgctt tatttattta caaaattttg ccataaagca 1320

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actgacttac cctttatagg tgatattagt gatatcaaaa ctgatatatt tttaagcaaa 1440

gatattaatg aagaaactga agttatagac tatccggaca atgtttcagt ggatcaagtt 1500

attctcagta agaatacctc agaacatgga caactagatt tattataccc tattattgaa 1560

ggtgagagtc aagtattacc gggagagaat caagtctttt atgataatag aactcaaaat 1620

gttgattatt tgaattctta ttattaccta gaatctcaaa aactaagtga taatgttgaa 1680

gattttactt ttacgacatc aattgaggaa gctttggata atagtggaaa agtatatact 1740

tactttccta aactagctga taaagtaaat acgggtgttc aaggtggttt atttttaatg 1800

tgggcaaatg atgtagttga agattttact acaaatattc taagaaaaga tacattagat 1860

aaaatatcag atgtatcagc tattattccc tatataggac ctgcattaaa tataagtaat 1920

tctgtaagaa ggggaaattt tactgaagca tttgcagtta ccggtgtaac tattttatta 1980

gaagcgtttc aagaatttac aatacctgca cttggtgcat ttgtgattta tagtaaggtt 2040

caagaaagaa acgagattat taaaactata gataattgtt tagaacaaag gattaaaaga 2100

tggaaagatt catatgaatg gatgatagga acgtggttat ccaggattac tactcaattt 2160

aataatataa gttatcaaat gtatgattct ttaaattatc aggcagatgc aatcaaagat 2220

aaaatagatt tagaatataa aaaatactca ggaagtgata aagaaaatat aaaaagtcaa 2280

gttgaaaatt taaaaaatag tttagatata aaaatctcgg aagcaatgaa taatataaat 2340

aaatttatac gagaatgttc tgtaacatac ttatttaaaa atatgctccc taaagtaatt 2400

gatgaattaa ataagtttga tttaaaaact aaaacagaat taattaatct tatagatagt 2460

cataatatta ttctagttgg tgaagtagat agattaaaag caaaagtaaa tgagagtttt 2520

gaaaatacaa taccctttaa tatttttca tatactaata attctttatt aaaagatata 2580

attaatgaat atttcaatag tattaatgat tcaaaaattt tgagcttaca aaacaaaaaa 2640

aatgctttag tggatacatc aggatataat gcagaagtga ggctagaagg tgatgttcaa 2700

gttaatacga tatatacaaa tgattttaaa ttaagtagtt caggagataa aattatagta 2760

aatttaaata ataatatttt atatagcgct atttatgaga actctagtgt tagttttgg 2820

attaagatat ctaaagattt aactaattct cataatgaat atacaataat taatagtata 2880

aaacaaaatt ctgggtggaa attatgtatt aggaatggca atatagaatg gattttacaa 2940

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gatattaata gaaagtataa aagtttaatt tttgattata gtgaatcatt aagtcataca 3000

ggatatacaa ataaatggtt ttttgttact ataactaata atataatggg gtatatgaaa 3060

ctttatataa atggagaatt aaagcagagt gaaagaattg aagatttaaa tgaggttaag 3120

ttagataaaa ccatagtatt tggaatagat gagaatatag atgagaatca gatgctttgg 3180

attagagatt ttaatatttt ttctaaagaa ttaagcaatg aagatattaa tattgtatat 3240

gagggacaaa tattaagaaa tgttattaaa gattattggg gaaatccttt gaagtttgat 3300

acagaatatt atattattaa tgataattat atagataggt atatagcacc taaaagtaat 3360

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gcaatagaag gaagagagtg ttcaaaaaat tgtgtatatg cattaaaatt acagagtaat 3600

ttaggtaatt atggtatagg tatatttagt ataaaaaata ttgtatctca aaataaatat 3660

tgtagtcaaa ttttctctag ttttatgaaa aatacaatgc ttctagcaga tatatataaa 3720

ccttggagat tttcttttga aaatgcatac acgccagttg cagtaactaa ttatgagaca 3780

aaactattat caacttcatc tttttggaaa tttatttcta gggatccagg atgggtagag 3840

taa 3843

<210> SEQ ID NO 6
<211> LENGTH: 1280
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 6

Met Pro Ile Thr Ile Asn Asn Phe Asn Tyr Ser Asp Pro Val Asp Asn
1 5 10 15

Lys Asn Ile Leu Tyr Leu Asp Thr His Leu Asn Thr Leu Ala Asn Glu
20 25 30

Pro Glu Lys Ala Phe Arg Ile Ile Gly Asn Ile Trp Val Ile Pro Asp
35 40 45

Arg Phe Ser Arg Asp Ser Asn Pro Asn Leu Asn Lys Pro Pro Arg Val
50 55 60

Thr Ser Pro Lys Ser Gly Tyr Tyr Asp Pro Asn Tyr Leu Ser Thr Asp
65 70 75 80

Ser Glu Lys Asp Thr Phe Leu Lys Glu Ile Ile Lys Leu Phe Lys Arg
85 90 95

Ile Asn Ser Arg Glu Ile Gly Glu Glu Leu Ile Tyr Arg Leu Ala Thr
100 105 110

Asp Ile Pro Phe Pro Gly Asn Asn Asn Thr Pro Ile Asn Thr Phe Asp
115 120 125

Phe Asp Val Asp Phe Asn Ser Val Asp Val Lys Thr Arg Gln Gly Asn
130 135 140

Asn Trp Val Lys Thr Gly Ser Ile Asn Pro Ser Val Ile Ile Thr Gly
145 150 155 160

Pro Arg Glu Asn Ile Ile Asp Pro Glu Thr Ser Thr Phe Lys Leu Thr
165 170 175

Asn Asn Thr Phe Ala Ala Gln Glu Gly Phe Gly Ala Leu Ser Ile Ile
180 185 190

Ser Ile Ser Pro Arg Phe Met Leu Thr Tyr Ser Asn Ala Thr Asn Asn
195 200 205

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Val Gly Glu Gly Arg Phe Ser Lys Ser Glu Phe Cys Met Asp Pro Ile
    210                 215                 220

Leu Ile Leu Met His Glu Leu Asn His Ala Met His Asn Leu Tyr Gly
225                 230                 235                 240

Ile Ala Ile Pro Asn Asp Gln Arg Ile Ser Ser Val Thr Ser Asn Ile
                245                 250                 255

Phe Tyr Ser Gln Tyr Lys Val Lys Leu Glu Tyr Ala Glu Ile Tyr Ala
            260                 265                 270

Phe Gly Gly Pro Thr Ile Asp Leu Ile Pro Lys Ser Ala Arg Lys Tyr
        275                 280                 285

Phe Glu Glu Lys Ala Leu Asp Tyr Tyr Arg Ser Ile Ala Lys Arg Leu
    290                 295                 300

Asn Ser Ile Thr Thr Ala Asn Pro Ser Ser Phe Asn Lys Tyr Ile Gly
305                 310                 315                 320

Glu Tyr Lys Gln Lys Leu Ile Arg Lys Tyr Arg Phe Val Val Glu Ser
                325                 330                 335

Ser Gly Glu Val Ala Val Asp Arg Asn Lys Phe Ala Glu Leu Tyr Lys
            340                 345                 350

Glu Leu Thr Gln Ile Phe Thr Glu Phe Asn Tyr Ala Lys Ile Tyr Asn
        355                 360                 365

Val Gln Asn Arg Lys Ile Tyr Leu Ser Asn Val Tyr Thr Pro Val Thr
    370                 375                 380

Ala Asn Ile Leu Asp Asp Asn Val Tyr Asp Ile Gln Asn Gly Phe Asn
385                 390                 395                 400

Ile Pro Lys Ser Asn Leu Asn Val Leu Phe Met Gly Gln Asn Leu Ser
                405                 410                 415

Arg Asn Pro Ala Leu Arg Lys Val Asn Pro Glu Asn Met Leu Tyr Leu
            420                 425                 430

Phe Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr Asn
        435                 440                 445

Lys Thr Leu Asp Cys Arg Glu Leu Leu Val Lys Asn Thr Asp Leu Pro
    450                 455                 460

Phe Ile Gly Asp Ile Ser Asp Ile Lys Thr Asp Ile Phe Leu Ser Lys
465                 470                 475                 480

Asp Ile Asn Glu Glu Thr Glu Val Ile Asp Tyr Pro Asp Asn Val Ser
                485                 490                 495

Val Asp Gln Val Ile Leu Ser Lys Asn Thr Ser Glu His Gly Gln Leu
            500                 505                 510

Asp Leu Leu Tyr Pro Ile Ile Glu Gly Glu Ser Gln Val Leu Pro Gly
        515                 520                 525

Glu Asn Gln Val Phe Tyr Asp Asn Arg Thr Gln Asn Val Asp Tyr Leu
    530                 535                 540

Asn Ser Tyr Tyr Tyr Leu Glu Ser Gln Lys Leu Ser Asp Asn Val Glu
545                 550                 555                 560

Asp Phe Thr Phe Thr Thr Ser Ile Glu Glu Ala Leu Asp Asn Ser Gly
                565                 570                 575

Lys Val Tyr Thr Tyr Phe Pro Lys Leu Ala Asp Lys Val Asn Thr Gly
            580                 585                 590

Val Gln Gly Gly Leu Phe Leu Met Trp Ala Asn Asp Val Val Glu Asp
        595                 600                 605

Phe Thr Thr Asn Ile Leu Arg Lys Asp Thr Leu Asp Lys Ile Ser Asp
    610                 615                 620
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Val Ser Ala Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Ser Asn
625                 630                 635                 640

Ser Val Arg Arg Gly Asn Phe Thr Glu Ala Phe Ala Val Thr Gly Val
                645                 650                 655

Thr Ile Leu Leu Glu Ala Phe Gln Glu Phe Thr Ile Pro Ala Leu Gly
            660                 665                 670

Ala Phe Val Ile Tyr Ser Lys Val Gln Glu Arg Asn Glu Ile Ile Lys
        675                 680                 685

Thr Ile Asp Asn Cys Leu Glu Gln Arg Ile Lys Arg Trp Lys Asp Ser
    690                 695                 700

Tyr Glu Trp Met Ile Gly Thr Trp Leu Ser Arg Ile Thr Thr Gln Phe
705                 710                 715                 720

Asn Asn Ile Ser Tyr Gln Met Tyr Asp Ser Leu Asn Tyr Gln Ala Asp
                725                 730                 735

Ala Ile Lys Asp Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser
            740                 745                 750

Asp Lys Glu Asn Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu
        755                 760                 765

Asp Ile Lys Ile Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg
    770                 775                 780

Glu Cys Ser Val Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile
785                 790                 795                 800

Asp Glu Leu Asn Lys Phe Asp Leu Lys Thr Lys Thr Glu Leu Ile Asn
                805                 810                 815

Leu Ile Asp Ser His Asn Ile Ile Leu Val Gly Glu Val Asp Arg Leu
            820                 825                 830

Lys Ala Lys Val Asn Glu Ser Phe Glu Asn Thr Ile Pro Phe Asn Ile
        835                 840                 845

Phe Ser Tyr Thr Asn Asn Ser Leu Leu Lys Asp Ile Ile Asn Glu Tyr
    850                 855                 860

Phe Asn Ser Ile Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Lys Lys
865                 870                 875                 880

Asn Ala Leu Val Asp Thr Ser Gly Tyr Asn Ala Glu Val Arg Leu Glu
                885                 890                 895

Gly Asp Val Gln Val Asn Thr Ile Tyr Thr Asn Asp Phe Lys Leu Ser
            900                 905                 910

Ser Ser Gly Asp Lys Ile Ile Val Asn Leu Asn Asn Asn Ile Leu Tyr
        915                 920                 925

Ser Ala Ile Tyr Glu Asn Ser Ser Val Ser Phe Trp Ile Lys Ile Ser
    930                 935                 940

Lys Asp Leu Thr Asn Ser His Asn Glu Tyr Thr Ile Ile Asn Ser Ile
945                 950                 955                 960

Lys Gln Asn Ser Gly Trp Lys Leu Cys Ile Arg Asn Gly Asn Ile Glu
                965                 970                 975

Trp Ile Leu Gln Asp Ile Asn Arg Lys Tyr Lys Ser Leu Ile Phe Asp
            980                 985                 990

Tyr Ser Glu Ser Leu Ser His Thr  Gly Tyr Thr Asn Lys  Trp Phe Phe
        995                 1000                1005

Val Thr  Ile Thr Asn Asn Ile  Met Gly Tyr Met Lys  Leu Tyr Ile
    1010                1015                1020

Asn Gly  Glu Leu Lys Gln Ser  Glu Arg Ile Glu Asp  Leu Asn Glu
    1025                1030                1035

Val Lys  Leu Asp Lys Thr Ile  Val Phe Gly Ile Asp  Glu Asn Ile
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    1040                1045                1050

Asp Glu  Asn Gln Met Leu Trp  Ile Arg Asp Phe Asn  Ile Phe Ser
    1055                1060                1065

Lys Glu  Leu Ser Asn Glu Asp  Ile Asn Ile Val Tyr  Glu Gly Gln
    1070                1075                1080

Ile Leu  Arg Asn Val Ile Lys  Asp Tyr Trp Gly Asn  Pro Leu Lys
    1085                1090                1095

Phe Asp  Thr Glu Tyr Tyr Ile  Ile Asn Asp Asn Tyr  Ile Asp Arg
    1100                1105                1110

Tyr Ile  Ala Pro Lys Ser Asn  Ile Leu Val Leu Val  Gln Tyr Pro
    1115                1120                1125

Asp Arg  Ser Lys Leu Tyr Thr  Gly Asn Pro Ile Thr  Ile Lys Ser
    1130                1135                1140

Val Ser  Asp Lys Asn Pro Tyr  Ser Arg Ile Leu Asn  Gly Asp Asn
    1145                1150                1155

Ile Met  Phe His Met Leu Tyr  Asn Ser Gly Lys Tyr  Met Ile Ile
    1160                1165                1170

Arg Asp  Thr Asp Thr Ile Tyr  Ala Ile Glu Gly Arg  Glu Cys Ser
    1175                1180                1185

Lys Asn  Cys Val Tyr Ala Leu  Lys Leu Gln Ser Asn  Leu Gly Asn
    1190                1195                1200

Tyr Gly  Ile Gly Ile Phe Ser  Ile Lys Asn Ile Val  Ser Gln Asn
    1205                1210                1215

Lys Tyr  Cys Ser Gln Ile Phe  Ser Ser Phe Met Lys  Asn Thr Met
    1220                1225                1230

Leu Leu  Ala Asp Ile Tyr Lys  Pro Trp Arg Phe Ser  Phe Glu Asn
    1235                1240                1245

Ala Tyr  Thr Pro Val Ala Val  Thr Asn Tyr Glu Thr  Lys Leu Leu
    1250                1255                1260

Ser Thr  Ser Ser Phe Trp Lys  Phe Ile Ser Arg Asp  Pro Gly Trp
    1265                1270                1275

Val Glu
    1280
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<210> SEQ ID NO 7
<211> LENGTH: 3858
<212> TYPE: DNA
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 7

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tatttaagaa taccacaaaa taagttaatt actacacctg taaaagcttt tatgattact    120

caaaatattt gggtaatacc agaaagattt tcatcagata ctaatccaag tttaagtaaa    180

ccgcctagac ctacttcaaa gtatcaaagt tattatgatc ctagttattt atctactgat    240

gagcaaaaag atacattttt aaaagggatt ataaaattat ttaaaagaat taatgaaaga    300

gatataggaa aaaaattaat aaattattta gtagttggtt caccttttat gggagattca    360

agtacgcctg aagatacatt tgattttaca cgtcatacta ctaatattgc agttgaaaag    420

tttgaaaatg gtagttggaa agtaacaaat attataacac caagtgtatt gatatttgga    480

ccacttccta atatattaga ctatacagca tcccttacat tgcaaggaca acaatcaaat    540

ccatcatttg aagggtttgg aacattatct atactaaaag tagcacctga atttttgtta    600

acatttagtg atgtaacatc taatcaaagt tcagctgtat taggcaaatc tatattttgt    660
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atggatccag taatagcttt aatgcatgag ttaacacatt ctttgcatca attgtatgga 720

ataaatatac catctgataa aaggattcgt ccacaagtta gcgagggatt tttttctcaa 780

gatggaccca acgtacaatt tgaggaatta tacacatttg gaggatcaga tgttgaaata 840

atacctcaaa ttgaaagatt acaattaaga gaaaaagcat taggtcacta taaagatata 900

gcgaaaagac ttaataatat taataaaact attccttcta gttggagtag taatatagat 960

aaatataaaa aaatattttc tgaaaagtat aattttgata aagataatac aggaaatttt 1020

gttgtaaata ttgataaatt caatagctta tattcagact tgactaatgt tatgtcagaa 1080

gttgtttatt cttcgcaata taatgttaaa aacaggactc attatttttc aaagcattat 1140

ctacctgtat ttgcaaatat attagatgat aatatttata ctataataaa cggttttaat 1200

ttaacaacta aaggttttaa tatagaaaat tcgggtcaga atatagaaag gaatcctgca 1260

ctacaaaaac ttagttcaga aagtgtagta gatttgttta caaaagtatg tttaagatta 1320

acaagaaata gtagagatga ttcaacatgt attcaagtta aaaataatac attaccttat 1380

gtagctgata aagatagcat ttcacaagaa atatttgaaa gtcaaattat tacagatgag 1440

actaatgtag aaaattattc agataatttt tcattagatg aatctatttt agatgcaaaa 1500

gtccctacta atcctgaagc agtagatcca ctgttaccca atgttaatat ggaaccttta 1560

aatgttccag gtgaagaaga agtattttat gatgatatta ctaaagatgt tgattattta 1620

aactcttatt attatttgga agcccaaaaa ttaagtaata atgttgaaaa tattactctt 1680

acaacttcag ttgaagaagc attaggttat agcaataaga tatacacatt tttacctagc 1740

ttagctgaaa aagtgaataa aggtgttcaa gcaggtttat tcttaaattg ggcgaatgaa 1800

gtagttgagg attttactac aaatattatg aaaaaagata cattggataa aatatcagat 1860

gtatcagcca taattccata tataggacct gccttaaata taggaaattc agcattaagg 1920

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gagtttacaa tacctgcact cggtgtattt acctttttata gttctattca agaaagagag 2040

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tatcaaatgt atgattcttt gagttatcag gcagatgcaa tcaaagctaa aatagattta 2220

gaatataaaa aatactcagg aagtgataaa gaaaatataa aaagtcaagt tgaaaattta 2280

aaaaatagtt tagatgtaaa aatctcggaa gcaatgaata atataaataa atttatacga 2340

gaatgttctg taacatactt atttaaaaat atgctcccta aagtaattga tgaattaaat 2400

aagtttgatt taaaaactaa aacagaatta attaatctta tagatagtca taatattatt 2460

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ccctttaata ttttttcata tactaataat tctttattaa aagatatgat taatgaatat 2580

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cagaatgaaa atattgtata taatgctatg tatgaaagtt ttagtattag tttttggatt 2820

aggataaata aatgggtaag taatttacct ggatatacta taattgatag tgttaaaaat 2880

aactcaggtt ggagtatagg tattattagt aatttttag tgtttacttt aaaacaaaat 2940

gaaaatagtg aacaagatat aaactttagt tatgatatat caaagaatgc tgcgggatat 3000

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actataacat ttcaaatgaa taaaattcca aatactggct taattacctc agattctgat 3180

aacatcaata tgtggataag ggatttttat atctttgcta aagaattaga tgataaagat 3240

attaatatat tatttaatag cttgcaatat actaatgttg taaaagatta ttggggaaat 3300

gatttaagat atgataaaga atattacatg attaacgtaa attatatgaa tagatatatg 3360

tctaaaaaag gcaatggaat tgtttttaat acacgtaaaa ataataatga cttcaatgaa 3420

ggatataaaa ttataataaa aagaattaga ggaaatacaa atgatactag agtacgagga 3480

gaaaatgtat tatattttaa tactacaatt gataacaaac aatatagttt aggtatgtat 3540

aaaccttcta gaaatctagg gactgattta gttccactag gtgcattgga tcaaccaatg 3600

gatgagatac gtaaatatgg ttcgtttata atacaaccat gcaatacttt tgattactat 3660

gcatcacaat tatttttgtc aagtaatgca acaacaaata ggcttggaat actatcaatt 3720

ggtagttata gtttcaaact tggagatgac tattggttta atcacgaata tttaattcct 3780

gttataaaaa tagagcatta tgcttcatta ttagaatcaa catcaactca ttgggttttt 3840

gtacctgcaa gtgaataa 3858

<210> SEQ ID NO 8
<211> LENGTH: 1285
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 8

Met Thr Trp Pro Val Lys Asp Phe Asn Tyr Ser Asp Pro Val Asn Asp
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Asn Asp Ile Leu Tyr Leu Arg Ile Pro Gln Asn Lys Leu Ile Thr Thr
20 25 30

Pro Val Lys Ala Phe Met Ile Thr Gln Asn Ile Trp Val Ile Pro Glu
35 40 45

Arg Phe Ser Ser Asp Thr Asn Pro Ser Leu Ser Lys Pro Pro Arg Pro
50 55 60

Thr Ser Lys Tyr Gln Ser Tyr Tyr Asp Pro Ser Tyr Leu Ser Thr Asp
65 70 75 80

Glu Gln Lys Asp Thr Phe Leu Lys Gly Ile Ile Lys Leu Phe Lys Arg
85 90 95

Ile Asn Glu Arg Asp Ile Gly Lys Lys Leu Ile Asn Tyr Leu Val Val
100 105 110

Gly Ser Pro Phe Met Gly Asp Ser Ser Thr Pro Glu Asp Thr Phe Asp
115 120 125

Phe Thr Arg His Thr Thr Asn Ile Ala Val Glu Lys Phe Glu Asn Gly
130 135 140

Ser Trp Lys Val Thr Asn Ile Ile Thr Pro Ser Val Leu Ile Phe Gly
145 150 155 160

Pro Leu Pro Asn Ile Leu Asp Tyr Thr Ala Ser Leu Thr Leu Gln Gly
165 170 175

Gln Gln Ser Asn Pro Ser Phe Glu Gly Phe Gly Thr Leu Ser Ile Leu
180 185 190

Lys Val Ala Pro Glu Phe Leu Leu Thr Phe Ser Asp Val Thr Ser Asn
195 200 205

Gln Ser Ser Ala Val Leu Gly Lys Ser Ile Phe Cys Met Asp Pro Val
210 215 220

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Ile Ala Leu Met His Glu Leu Thr His Ser Leu His Gln Leu Tyr Gly
225                 230                 235                 240

Ile Asn Ile Pro Ser Asp Lys Arg Ile Arg Pro Gln Val Ser Glu Gly
                245                 250                 255

Phe Phe Ser Gln Asp Gly Pro Asn Val Gln Phe Glu Glu Leu Tyr Thr
            260                 265                 270

Phe Gly Gly Ser Asp Val Glu Ile Ile Pro Gln Ile Glu Arg Leu Gln
        275                 280                 285

Leu Arg Glu Lys Ala Leu Gly His Tyr Lys Asp Ile Ala Lys Arg Leu
    290                 295                 300

Asn Asn Ile Asn Lys Thr Ile Pro Ser Ser Trp Ser Ser Asn Ile Asp
305                 310                 315                 320

Lys Tyr Lys Lys Ile Phe Ser Glu Lys Tyr Asn Phe Asp Lys Asp Asn
                325                 330                 335

Thr Gly Asn Phe Val Val Asn Ile Asp Lys Phe Asn Ser Leu Tyr Ser
            340                 345                 350

Asp Leu Thr Asn Val Met Ser Glu Val Val Tyr Ser Ser Gln Tyr Asn
        355                 360                 365

Val Lys Asn Arg Thr His Tyr Phe Ser Lys His Tyr Leu Pro Val Phe
    370                 375                 380

Ala Asn Ile Leu Asp Asp Asn Ile Tyr Thr Ile Ile Asn Gly Phe Asn
385                 390                 395                 400

Leu Thr Thr Lys Gly Phe Asn Ile Glu Asn Ser Gly Gln Asn Ile Glu
                405                 410                 415

Arg Asn Pro Ala Leu Gln Lys Leu Ser Ser Glu Ser Val Val Asp Leu
            420                 425                 430

Phe Thr Lys Val Cys Leu Arg Leu Thr Arg Asn Ser Arg Asp Asp Ser
        435                 440                 445

Thr Cys Ile Gln Val Lys Asn Asn Thr Leu Pro Tyr Val Ala Asp Lys
    450                 455                 460

Asp Ser Ile Ser Gln Glu Ile Phe Glu Ser Gln Ile Ile Thr Asp Glu
465                 470                 475                 480

Thr Asn Val Glu Asn Tyr Ser Asp Asn Phe Ser Leu Asp Glu Ser Ile
                485                 490                 495

Leu Asp Ala Lys Val Pro Thr Asn Pro Glu Ala Val Asp Pro Leu Leu
            500                 505                 510

Pro Asn Val Asn Met Glu Pro Leu Asn Val Pro Gly Glu Glu Glu Val
        515                 520                 525

Phe Tyr Asp Asp Ile Thr Lys Asp Val Asp Tyr Leu Asn Ser Tyr Tyr
    530                 535                 540

Tyr Leu Glu Ala Gln Lys Leu Ser Asn Asn Val Glu Asn Ile Thr Leu
545                 550                 555                 560

Thr Thr Ser Val Glu Glu Ala Leu Gly Tyr Ser Asn Lys Ile Tyr Thr
                565                 570                 575

Phe Leu Pro Ser Leu Ala Glu Lys Val Asn Lys Gly Val Gln Ala Gly
            580                 585                 590

Leu Phe Leu Asn Trp Ala Asn Glu Val Val Glu Asp Phe Thr Thr Asn
        595                 600                 605

Ile Met Lys Lys Asp Thr Leu Asp Lys Ile Ser Asp Val Ser Ala Ile
    610                 615                 620

Ile Pro Tyr Ile Gly Pro Ala Leu Asn Ile Gly Asn Ser Ala Leu Arg
625                 630                 635                 640
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Gly Asn Phe Lys Gln Ala Phe Ala Thr Ala Gly Val Ala Phe Leu Leu
                645                 650                 655

Glu Gly Phe Pro Glu Phe Thr Ile Pro Ala Leu Gly Val Phe Thr Phe
            660                 665                 670

Tyr Ser Ser Ile Gln Glu Arg Glu Lys Ile Ile Lys Thr Ile Glu Asn
        675                 680                 685

Cys Leu Glu Gln Arg Val Lys Arg Trp Lys Asp Ser Tyr Gln Trp Met
    690                 695                 700

Val Ser Asn Trp Leu Ser Arg Ile Thr Thr Arg Phe Asn His Ile Ser
705                 710                 715                 720

Tyr Gln Met Tyr Asp Ser Leu Ser Tyr Gln Ala Asp Ala Ile Lys Ala
                725                 730                 735

Lys Ile Asp Leu Glu Tyr Lys Lys Tyr Ser Gly Ser Asp Lys Glu Asn
            740                 745                 750

Ile Lys Ser Gln Val Glu Asn Leu Lys Asn Ser Leu Asp Val Lys Ile
        755                 760                 765

Ser Glu Ala Met Asn Asn Ile Asn Lys Phe Ile Arg Glu Cys Ser Val
    770                 775                 780

Thr Tyr Leu Phe Lys Asn Met Leu Pro Lys Val Ile Asp Glu Leu Asn
785                 790                 795                 800

Lys Phe Asp Leu Lys Thr Lys Thr Glu Leu Ile Asn Leu Ile Asp Ser
                805                 810                 815

His Asn Ile Ile Leu Val Gly Glu Val Asp Arg Leu Lys Ala Lys Val
            820                 825                 830

Asn Glu Ser Phe Glu Asn Thr Ile Pro Phe Asn Ile Phe Ser Tyr Thr
        835                 840                 845

Asn Asn Ser Leu Leu Lys Asp Met Ile Asn Glu Tyr Phe Asn Ser Ile
    850                 855                 860

Asn Asp Ser Lys Ile Leu Ser Leu Gln Asn Lys Lys Asn Thr Leu Met
865                 870                 875                 880

Asp Thr Ser Gly Tyr Asn Ala Glu Val Arg Val Glu Gly Asn Val Gln
                885                 890                 895

Leu Asn Pro Ile Phe Pro Phe Asp Phe Lys Leu Gly Ser Ser Gly Asp
            900                 905                 910

Asp Arg Gly Lys Val Ile Val Thr Gln Asn Glu Asn Ile Val Tyr Asn
        915                 920                 925

Ala Met Tyr Glu Ser Phe Ser Ile Ser Phe Trp Ile Arg Ile Asn Lys
    930                 935                 940

Trp Val Ser Asn Leu Pro Gly Tyr Thr Ile Ile Asp Ser Val Lys Asn
945                 950                 955                 960

Asn Ser Gly Trp Ser Ile Gly Ile Ile Ser Asn Phe Leu Val Phe Thr
                965                 970                 975

Leu Lys Gln Asn Glu Asn Ser Glu Gln Asp Ile Asn Phe Ser Tyr Asp
            980                 985                 990

Ile Ser Lys Asn Ala Ala Gly Tyr  Asn Lys Trp Phe Phe  Val Thr Ile
        995                 1000                1005

Thr Thr  Asn Met Met Gly Asn  Met Met Ile Tyr Ile  Asn Gly Lys
    1010                1015                1020

Leu Ile  Asp Thr Ile Lys Val  Lys Glu Leu Thr Gly  Ile Asn Phe
    1025                1030                1035

Ser Lys  Thr Ile Thr Phe Gln  Met Asn Lys Ile Pro  Asn Thr Gly
    1040                1045                1050

Leu Ile  Thr Ser Asp Ser Asp  Asn Ile Asn Met Trp  Ile Arg Asp
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    1055                1060                1065

Phe Tyr  Ile Phe Ala Lys Glu  Leu Asp Asp Lys Asp  Ile Asn Ile
    1070                1075                1080

Leu Phe  Asn Ser Leu Gln Tyr  Thr Asn Val Val Lys  Asp Tyr Trp
    1085                1090                1095

Gly Asn  Asp Leu Arg Tyr Asp  Lys Glu Tyr Tyr Met  Ile Asn Val
    1100                1105                1110

Asn Tyr  Met Asn Arg Tyr Met  Ser Lys Lys Gly Asn  Gly Ile Val
    1115                1120                1125

Phe Asn  Thr Arg Lys Asn Asn  Asn Asp Phe Asn Glu  Gly Tyr Lys
    1130                1135                1140

Ile Ile  Ile Lys Arg Ile Arg  Gly Asn Thr Asn Asp  Thr Arg Val
    1145                1150                1155

Arg Gly  Glu Asn Val Leu Tyr  Phe Asn Thr Thr Ile  Asp Asn Lys
    1160                1165                1170

Gln Tyr  Ser Leu Gly Met Tyr  Lys Pro Ser Arg Asn  Leu Gly Thr
    1175                1180                1185

Asp Leu  Val Pro Leu Gly Ala  Leu Asp Gln Pro Met  Asp Glu Ile
    1190                1195                1200

Arg Lys  Tyr Gly Ser Phe Ile  Ile Gln Pro Cys Asn  Thr Phe Asp
    1205                1210                1215

Tyr Tyr  Ala Ser Gln Leu Phe  Leu Ser Ser Asn Ala  Thr Thr Asn
    1220                1225                1230

Arg Leu  Gly Ile Leu Ser Ile  Gly Ser Tyr Ser Phe  Lys Leu Gly
    1235                1240                1245

Asp Asp  Tyr Trp Phe Asn His  Glu Tyr Leu Ile Pro  Val Ile Lys
    1250                1255                1260

Ile Glu  His Tyr Ala Ser Leu  Leu Glu Ser Thr Ser  Thr His Trp
    1265                1270                1275

Val Phe  Val Pro Ala Ser Glu
    1280                1285


<210> SEQ ID NO 9
<211> LENGTH: 3756
<212> TYPE: DNA
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 9

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ataattccag agagaaatgt aattggtaca acccccccaag attttcatcc gcctacttca    180

ttaaaaaatg gagatagtag ttattatgac cctaattatt tacaaagtga tgaagaaaag    240

gatagatttt taaaaatagt cacaaaaata tttaatagaa taaataataa tctttcagga    300

gggattttat tagaagaact gtcaaaagct aatccatatt tagggaatga taatactcca    360

gataatcaat tccatattgg tgatgcatca gcagttgaga ttaaattctc aaatggtagc    420

caagacatac tattacctaa tgttattata atgggagcag agcctgattt atttgaaact    480

aacagttcca atatttctct aagaaataat tatatgccaa gcaatcaccg ttttggatca    540

atagctatag taacattctc acctgaatat tcttttagat ttaatgataa ttgtatgaat    600

gaatttattc aagatcctgc tcttacatta atgcatgaat taatacattc attacatgga    660

ctatatgggg ctaaagggat tactacaaag tatactataa cacaaaaaca aaatccccta    720
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ataacaaata taagaggtac aaatattgaa gaattcttaa cttttggagg tactgattta 780

aacattatta ctagtgctca gtccaatgat atctatacta atcttctagc tgattataaa 840

aaaatagcgt ctaaacttag caaagtacaa gtatctaatc cactacttaa tccttataaa 900

gatgtttttg aagcaaagta tggattagat aaagatgcta gcggaattta ttcggtaaat 960

ataaacaaat ttaatgatat ttttaaaaaa ttatacagct ttacggaatt tgatttacga 1020

actaaatttc aagttaaatg taggcaaact tatattggac agtataaata cttcaaactt 1080

tcaaacttgt taaatgattc tatttataat atatcagaag gctataatat aaataattta 1140

aaggtaaatt ttagaggaca gaatgcaaat ttaaatccta gaattattac accaattaca 1200

ggtagaggac tagtaaaaaa aatcattaga ttttgtaaaa atattgtttc tgtaaaaggc 1260

ataaggaaat caatatgtat cgaaataaat aatggtgagt tatttttgt ggcttccgag 1320

aatagttata atgatgataa tataaatact cctaaagaaa ttgacgatac agtaacttca 1380

aataataatt atgaaaatga tttagatcag gttattttaa attttaatag tgaatcagca 1440

cctggacttt cagatgaaaa attaaattta actatccaaa atgatgctta tataccaaaa 1500

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ttaattccta caattttagt attcacgata aaatcttttt taggttcatc tgataataaa 1980

aataaagtta ttaaagcaat aaataatgca ttgaaagaaa gagatgaaaa atggaaagaa 2040

gtatatagtt ttatagtatc gaattggatg actaaaatta atacacaatt taataaaaga 2100

aaagaacaaa tgtatcaagc tttacaaaat caagtaaatg caattaaaac aataatagaa 2160

tctaagtata atagttatac tttagaggaa aaaaatgagc ttacaaataa atatgatatt 2220

aagcaaatag aaaatgaact taatcaaaag gtttctatag caatgaataa tatagacagg 2280

ttcttaactg aaagttctat atcctattta atgaaaataa taaatgaagt aaaaattaat 2340

aaattaagag aatatgatga gaatgtcaaa acgtatttat tgaattatat tatacaacat 2400

ggatcaatct tgggagagag tcagcaagaa ctaaattcta tggtaactga taccctaaat 2460

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aataaattct ttaagagaat taaaagtagt tcagttttaa atatgagata taaaaatgat 2580

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tatccaacta ataaaaatca atttggaata tataatgata aacttagtga agttaatata 2700

tctcaaaatg attacattat atatgataat aaatataaaa attttagtat tagtttttgg 2760

gtaagaattc ctaactatga taataagata gtaaatgtta ataatgaata cactataata 2820

aattgtatga gagataataa ttcaggatgg aaagtatctc ttaatcataa tgaaataatt 2880

tggacattcg aagataatcg aggaattaat caaaaattag catttaacta tggtaacgca 2940

aatggtattt ctgattatat aaataagtgg atttttgtaa ctataactaa tgatagatta 3000

ggagattcta aactttatat taatggaaat ttaatagatc aaaaatcaat tttaaattta 3060

ggtaatattc atgttagtga caatatatta tttaaaatag ttaattgtag ttatacaaga 3120

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tatattggta ttagatattt taatattttt gataaagaat tagatgaaac agaaattcaa 3180

actttatata gcaatgaacc taatacaaat attttgaagg atttttgggg aaattatttg 3240

ctttatgaca aagaatacta tttattaaat gtgttaaaac caaataactt tattgatagg 3300

agaaaagatt ctactttaag cattaataat ataagaagca ctattctttt agctaataga 3360

ttatatagtg gaataaaagt taaaatacaa agagttaata atagtagtac taacgataat 3420

cttgttagaa agaatgatca ggtatatatt aattttgtag ccagcaaaac tcacttattt 3480

ccattatatg ctgatacagc taccacaaat aaagagaaaa caataaaaat atcatcatct 3540

ggcaatagat ttaatcaagt agtagttatg aattcagtag gaaattgtac aatgaatttt 3600

aaaaataata atggaaataa tattgggttg ttaggtttca aggcagatac tgtcgttgct 3660

agtacttggt attatacaca tatgagagat catacaaaca gcaatggatg tttttggaac 3720

tttatttctg aagaacatgg atggcaagaa aaataa 3756

<210> SEQ ID NO 10
<211> LENGTH: 1251
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 10

Met Pro Lys Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp Arg
1 5 10 15

Thr Ile Leu Tyr Ile Lys Pro Gly Gly Cys Gln Glu Phe Tyr Lys Ser
20 25 30

Phe Asn Ile Met Lys Asn Ile Trp Ile Ile Pro Glu Arg Asn Val Ile
35 40 45

Gly Thr Thr Pro Gln Asp Phe His Pro Pro Thr Ser Leu Lys Asn Gly
50 55 60

Asp Ser Ser Tyr Tyr Asp Pro Asn Tyr Leu Gln Ser Asp Glu Glu Lys
65 70 75 80

Asp Arg Phe Leu Lys Ile Val Thr Lys Ile Phe Asn Arg Ile Asn Asn
85 90 95

Asn Leu Ser Gly Gly Ile Leu Leu Glu Glu Leu Ser Lys Ala Asn Pro
100 105 110

Tyr Leu Gly Asn Asp Asn Thr Pro Asp Asn Gln Phe His Ile Gly Asp
115 120 125

Ala Ser Ala Val Glu Ile Lys Phe Ser Asn Gly Ser Gln Asp Ile Leu
130 135 140

Leu Pro Asn Val Ile Ile Met Gly Ala Glu Pro Asp Leu Phe Glu Thr
145 150 155 160

Asn Ser Ser Asn Ile Ser Leu Arg Asn Asn Tyr Met Pro Ser Asn His
165 170 175

Arg Phe Gly Ser Ile Ala Ile Val Thr Phe Ser Pro Glu Tyr Ser Phe
180 185 190

Arg Phe Asn Asp Asn Cys Met Asn Glu Phe Ile Gln Asp Pro Ala Leu
195 200 205

Thr Leu Met His Glu Leu Ile His Ser Leu His Gly Leu Tyr Gly Ala
210 215 220

Lys Gly Ile Thr Thr Lys Tyr Thr Ile Thr Gln Lys Gln Asn Pro Leu
225 230 235 240

Ile Thr Asn Ile Arg Gly Thr Asn Ile Glu Glu Phe Leu Thr Phe Gly
245 250 255

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Gly Thr Asp Leu Asn Ile Ile Thr Ser Ala Gln Ser Asn Asp Ile Tyr
            260                 265                 270

Thr Asn Leu Leu Ala Asp Tyr Lys Lys Ile Ala Ser Lys Leu Ser Lys
        275                 280                 285

Val Gln Val Ser Asn Pro Leu Leu Asn Pro Tyr Lys Asp Val Phe Glu
    290                 295                 300

Ala Lys Tyr Gly Leu Asp Lys Asp Ala Ser Gly Ile Tyr Ser Val Asn
305                 310                 315                 320

Ile Asn Lys Phe Asn Asp Ile Phe Lys Lys Leu Tyr Ser Phe Thr Glu
                325                 330                 335

Phe Asp Leu Arg Thr Lys Phe Gln Val Lys Cys Arg Gln Thr Tyr Ile
            340                 345                 350

Gly Gln Tyr Lys Tyr Phe Lys Leu Ser Asn Leu Leu Asn Asp Ser Ile
        355                 360                 365

Tyr Asn Ile Ser Glu Gly Tyr Asn Ile Asn Asn Leu Lys Val Asn Phe
    370                 375                 380

Arg Gly Gln Asn Ala Asn Leu Asn Pro Arg Ile Ile Thr Pro Ile Thr
385                 390                 395                 400

Gly Arg Gly Leu Val Lys Lys Ile Ile Arg Phe Cys Lys Asn Ile Val
                405                 410                 415

Ser Val Lys Gly Ile Arg Lys Ser Ile Cys Ile Glu Ile Asn Asn Gly
            420                 425                 430

Glu Leu Phe Phe Val Ala Ser Glu Asn Ser Tyr Asn Asp Asp Asn Ile
        435                 440                 445

Asn Thr Pro Lys Glu Ile Asp Asp Thr Val Thr Ser Asn Asn Asn Tyr
    450                 455                 460

Glu Asn Asp Leu Asp Gln Val Ile Leu Asn Phe Asn Ser Glu Ser Ala
465                 470                 475                 480

Pro Gly Leu Ser Asp Glu Lys Leu Asn Leu Thr Ile Gln Asn Asp Ala
                485                 490                 495

Tyr Ile Pro Lys Tyr Asp Ser Asn Gly Thr Ser Asp Ile Glu Gln His
            500                 505                 510

Asp Val Asn Glu Leu Asn Val Phe Phe Tyr Leu Asp Ala Gln Lys Val
        515                 520                 525

Pro Glu Gly Glu Asn Asn Val Asn Leu Thr Ser Ser Ile Asp Thr Ala
    530                 535                 540

Leu Leu Glu Gln Pro Lys Ile Tyr Thr Phe Phe Ser Ser Glu Phe Ile
545                 550                 555                 560

Asn Asn Val Asn Lys Pro Val Gln Ala Ala Leu Phe Val Ser Trp Ile
                565                 570                 575

Gln Gln Val Leu Val Asp Phe Thr Thr Glu Ala Asn Gln Lys Ser Thr
            580                 585                 590

Val Asp Lys Ile Ala Asp Ile Ser Ile Val Val Pro Tyr Ile Gly Leu
        595                 600                 605

Ala Leu Asn Ile Gly Asn Glu Ala Gln Lys Gly Asn Phe Lys Asp Ala
    610                 615                 620

Leu Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Glu Pro Glu Leu
625                 630                 635                 640

Leu Ile Pro Thr Ile Leu Val Phe Thr Ile Lys Ser Phe Leu Gly Ser
                645                 650                 655

Ser Asp Asn Lys Asn Lys Val Ile Lys Ala Ile Asn Asn Ala Leu Lys
            660                 665                 670

Glu Arg Asp Glu Lys Trp Lys Glu Val Tyr Ser Phe Ile Val Ser Asn
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        675                 680                 685

Trp Met Thr Lys Ile Asn Thr Gln Phe Asn Lys Arg Lys Glu Gln Met
    690                 695                 700

Tyr Gln Ala Leu Gln Asn Gln Val Asn Ala Ile Lys Thr Ile Ile Glu
705                 710                 715                 720

Ser Lys Tyr Asn Ser Tyr Thr Leu Glu Glu Lys Asn Glu Leu Thr Asn
                725                 730                 735

Lys Tyr Asp Ile Lys Gln Ile Glu Asn Glu Leu Asn Gln Lys Val Ser
            740                 745                 750

Ile Ala Met Asn Asn Ile Asp Arg Phe Leu Thr Glu Ser Ser Ile Ser
        755                 760                 765

Tyr Leu Met Lys Ile Ile Asn Glu Val Lys Ile Asn Lys Leu Arg Glu
    770                 775                 780

Tyr Asp Glu Asn Val Lys Thr Tyr Leu Leu Asn Tyr Ile Ile Gln His
785                 790                 795                 800

Gly Ser Ile Leu Gly Glu Ser Gln Gln Glu Leu Asn Ser Met Val Thr
                805                 810                 815

Asp Thr Leu Asn Asn Ser Ile Pro Phe Lys Leu Ser Ser Tyr Thr Asp
            820                 825                 830

Asp Lys Ile Leu Ile Ser Tyr Phe Asn Lys Phe Phe Lys Arg Ile Lys
        835                 840                 845

Ser Ser Ser Val Leu Asn Met Arg Tyr Lys Asn Asp Lys Tyr Val Asp
    850                 855                 860

Thr Ser Gly Tyr Asp Ser Asn Ile Asn Ile Asn Gly Asp Val Tyr Lys
865                 870                 875                 880

Tyr Pro Thr Asn Lys Asn Gln Phe Gly Ile Tyr Asn Asp Lys Leu Ser
                885                 890                 895

Glu Val Asn Ile Ser Gln Asn Asp Tyr Ile Ile Tyr Asp Asn Lys Tyr
            900                 905                 910

Lys Asn Phe Ser Ile Ser Phe Trp Val Arg Ile Pro Asn Tyr Asp Asn
        915                 920                 925

Lys Ile Val Asn Val Asn Asn Glu Tyr Thr Ile Ile Asn Cys Met Arg
    930                 935                 940

Asp Asn Asn Ser Gly Trp Lys Val Ser Leu Asn His Asn Glu Ile Ile
945                 950                 955                 960

Trp Thr Phe Glu Asp Asn Arg Gly Ile Asn Gln Lys Leu Ala Phe Asn
                965                 970                 975

Tyr Gly Asn Ala Asn Gly Ile Ser Asp Tyr Ile Asn Lys Trp Ile Phe
            980                 985                 990

Val Thr Ile Thr Asn Asp Arg Leu  Gly Asp Ser Lys Leu  Tyr Ile Asn
        995                 1000                1005

Gly Asn  Leu Ile Asp Gln Lys  Ser Ile Leu Asn Leu  Gly Asn Ile
    1010                1015                1020

His Val  Ser Asp Asn Ile Leu  Phe Lys Ile Val Asn  Cys Ser Tyr
    1025                1030                1035

Thr Arg  Tyr Ile Gly Ile Arg  Tyr Phe Asn Ile Phe  Asp Lys Glu
    1040                1045                1050

Leu Asp  Glu Thr Glu Ile Gln  Thr Leu Tyr Ser Asn  Glu Pro Asn
    1055                1060                1065

Thr Asn  Ile Leu Lys Asp Phe  Trp Gly Asn Tyr Leu  Leu Tyr Asp
    1070                1075                1080

Lys Glu  Tyr Tyr Leu Leu Asn  Val Leu Lys Pro Asn  Asn Phe Ile
    1085                1090                1095
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Asp Arg  Arg Lys Asp Ser Thr  Leu Ser Ile Asn Asn  Ile Arg Ser
    1100                1105                1110

Thr Ile  Leu Leu Ala Asn Arg  Leu Tyr Ser Gly Ile  Lys Val Lys
    1115                1120                1125

Ile Gln  Arg Val Asn Asn Ser  Ser Thr Asn Asp Asn  Leu Val Arg
    1130                1135                1140

Lys Asn  Asp Gln Val Tyr Ile  Asn Phe Val Ala Ser  Lys Thr His
    1145                1150                1155

Leu Phe  Pro Leu Tyr Ala Asp  Thr Ala Thr Thr Asn  Lys Glu Lys
    1160                1165                1170

Thr Ile  Lys Ile Ser Ser Ser  Gly Asn Arg Phe Asn  Gln Val Val
    1175                1180                1185

Val Met  Asn Ser Val Gly Asn  Cys Thr Met Asn Phe  Lys Asn Asn
    1190                1195                1200

Asn Gly  Asn Asn Ile Gly Leu  Leu Gly Phe Lys Ala  Asp Thr Val
    1205                1210                1215

Val Ala  Ser Thr Trp Tyr Tyr  Thr His Met Arg Asp  His Thr Asn
    1220                1225                1230

Ser Asn  Gly Cys Phe Trp Asn  Phe Ile Ser Glu Glu  His Gly Trp
    1235                1240                1245

Gln Glu  Lys
    1250
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<210> SEQ ID NO 11
<211> LENGTH: 3843
<212> TYPE: DNA
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 11

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atgccagttg taataaatag ttttaattat aatgaccctg ttaatgatga gacaatttta     60

tacatgcaga aaccatatga agaaagaagt agaaaatatt ataaagcttt tgagattatg    120

cctaatgttt ggataatgcc tgagagagat acaataggaa ctaagcctga tgagtttcag    180

gtgccggatt cattaaagaa cggaagtagt gcttattatg atcctaatta tttaaccact    240

gatgctgaaa aagatagata tttaaaaaca atgataaaat tatttaatag aattaatagt    300

aatcctacag ggaaagtttt gttagaagaa gtatcaaatg ctagaccata tttaggagat    360

gatgacacgc taattaatga attccttcca gttaatgtaa ctacaagtgt taatataaaa    420

ttttcaactg atgttgaaag ttcaataata tcgaatcttc ttgtattggg agcaggacct    480

gatatattta aagcttactg tacccccctt gtaaggttta ataagtcaga taaattaatt    540

gaaccaagta atcatggttt tggatcaatt aatatcttga cattttcacc tgagtatgaa    600

catattttta atgatattag tggagggaat cataatagta cagaatcatt tattgcagat    660

cctgcaattt cactagctca tgaattgata catgcactac atggattata cggggctaag    720

gcagttactc ataaagagtc tctagtagca gagcgaggac ctcttatgat agccgaaaag    780

cccataaggc tagaagaatt tttaactttt ggaggtgagg atttaaatat cattcctagt    840

gctatgaagg aaaaaatata taacgatctt ttagctaact atgaaaaaat agctactaga    900

cttagagaag ttaatacggc tcctcctgga tatgatatta atgaatataa agattatttt    960

caatggaagt atggactaga tagaaatgca gatggaagtt atactgtgaa tagaaataaa   1020

tttaatgaaa tttataaaaa attatatagc tttacagaga ttgacttagc aaataaattt   1080

aaagtaaaat gtagaaatac ttattttatt aaatatggat ttgtaaaagt tccaaatttg   1140
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ttagatgatg atatttatac tgtatcagag gggtttaata taggtaattt agcagtaaac 1200

aatcgcggac aaaatataaa tttaaatcct aaaattattg attccattcc agataaaggt 1260

ttagtggaaa agattattaa attttgtaag agcattattc ctagaaaagg tacgaagcag 1320

tcaccgtcac tatgcattag agtaaataat agggagttat tttttgtagc ttcagaaagt 1380

agctataatg aaagtgatat taatacacct aaagaaattg acgatacaac aaatctaaat 1440

aataattata gaaataattt agatgaagtt attttagatt ataatagtga gacaatacct 1500

caaatatcaa atcgaacatt aaatacactt gtacaagaca atagttatgt gccaagatat 1560

gattctaatg gaacaagtga aatagaggaa tatgatgttg ttgactttaa tgtatttttc 1620

tatttacatg cacaaaaagt accagaaggt gaaaccaata taagtttaac ttcttcaatt 1680

gatacagcat tattagaaga atccaaagta tatacatttt tttcttcaga gtttatcgat 1740

actatcaata aacctgtaaa tgcagcacta tttatagatt ggataagcaa agtaataaga 1800

gattttacca ctgaagctac acaaaaaagt actgttgata agattgcaga catatcttta 1860

attgtaccct atgtaggtct tgctttgaat atagttattg aggcagaaaa aggaaatttt 1920

gaggaggcat ttgaattatt aggagcgggt attttattag aatttgtgcc agagcttaca 1980

attcctgtaa ttttagtgtt tacgataaaa tcctatatag attcatatga gaataaaaat 2040

aaagcaatta aagcaataaa taattcatta atcgaaagag aagcaaagtg gaaagaaata 2100

tatagttgga tagtatcaaa ttggcttact agaattaata cgcaatttaa taaaagaaaa 2160

gagcaaatgt atcaggcttt acaaaatcaa gtagatgcaa taaaaacagc aatagaatat 2220

aaatataata attatacttc agatgagaaa aatagacttg aatctaaata taatatcaat 2280

aatatagaag aagaattgaa taaaaaagtt tctttagcaa tgaaaaatat agaaagattt 2340

atgacagaaa gttctatatc ttatttaatg aaattaataa atgaagccga agttggtaaa 2400

ttaaaagaat atgataaaca tgttaagagc gatttattag actatattct ctaccataaa 2460

ttaatcttag gagagcagac aaaggaatta attgatttgg tgactagtac tttgaatagt 2520

agtattccat ttgaactttc ttcatatact aatgataaaa ttctaattat atattttaat 2580

agattatata aaaaaattaa agatagttct attttagata tgcgatatga aaataataaa 2640

tttatagata tctctggata tggttcaaat ataagcatta atggaaacgt atatatttat 2700

tcaacaaata gaaatcaatt tggaatatat agtggtaggc ttagtgaagt taatatagct 2760

caaaataatg atattatata caatagtaga tatcaaaatt ttagtattag tttctgggta 2820

accattccta aacactacag acctatgaat cgtaatcggg aatacactat aataaattgt 2880

atggggaata ataattcggg atggaaaata tcacttagaa ctattagaga ttgtgaaata 2940

atttggactt tacaagatac ttccggaaat aaggaaaaat taatttttag gtatgaagaa 3000

cttgctagta tatctgatta tataaataaa tggatttttg taactattac taataataga 3060

ttaggcaatt ctagaattta catcaatgga aatttaatag ttgaaaaatc aatttcgaat 3120

ttaggtgata ttcatgttag tgataatata ttatttaaaa ttgttggttg tgatgatgaa 3180

acgtatgttg gtataagata ttttaaagtt tttaatacgg aattagataa aacagaaatt 3240

gagactttat atagtaatga gccagatcca agtatcttaa aagactattg gggaaattat 3300

ttgctatata ataaaaaata ttatttattc aatttactaa gaaaagataa gtatattact 3360

cggaattcag gcattttaaa tattaatcaa caaagaggtg ttactggagg catatctgtt 3420

tttttgaact ataaattata tgaaggagta gaagttatta taagaaaaaa tgctcctata 3480

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gatatatcta atacagataa ttttgttaga aaaaacgatc tagcatacat taatgtagta 3540

gatcatggtg tagaatatcg gttatatgct gatatatcaa ttacaaaatc agagaaaata 3600

ataaaattaa taagaacatc taatccaaac gatagcttag gtcaaattat agttatggat 3660

tcaataggaa ataattgcac aatgaatttt caaaacaatg atgggagcaa tataggatta 3720

ctaggttttc attcagatga tttggttgct agtagttggt attataacca tatacgaaga 3780

aacactagca gtaatggatg cttttggagt tttatttcta aagagcatgg ttggaaagaa 3840

taa 3843

<210> SEQ ID NO 12
<211> LENGTH: 1280
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 12

Met Pro Val Val Ile Asn Ser Phe Asn Tyr Asn Asp Pro Val Asn Asp
1 5 10 15

Glu Thr Ile Leu Tyr Met Gln Lys Pro Tyr Glu Glu Arg Ser Arg Lys
20 25 30

Tyr Tyr Lys Ala Phe Glu Ile Met Pro Asn Val Trp Ile Met Pro Glu
35 40 45

Arg Asp Thr Ile Gly Thr Lys Pro Asp Glu Phe Gln Val Pro Asp Ser
50 55 60

Leu Lys Asn Gly Ser Ser Ala Tyr Tyr Asp Pro Asn Tyr Leu Thr Thr
65 70 75 80

Asp Ala Glu Lys Asp Arg Tyr Leu Lys Thr Met Ile Lys Leu Phe Asn
85 90 95

Arg Ile Asn Ser Asn Pro Thr Gly Lys Val Leu Leu Glu Glu Val Ser
100 105 110

Asn Ala Arg Pro Tyr Leu Gly Asp Asp Thr Leu Ile Asn Glu Phe
115 120 125

Leu Pro Val Asn Val Thr Thr Ser Val Asn Ile Lys Phe Ser Thr Asp
130 135 140

Val Glu Ser Ser Ile Ile Ser Asn Leu Leu Val Leu Gly Ala Gly Pro
145 150 155 160

Asp Ile Phe Lys Ala Tyr Cys Thr Pro Leu Val Arg Phe Asn Lys Ser
165 170 175

Asp Lys Leu Ile Glu Pro Ser Asn His Gly Phe Gly Ser Ile Asn Ile
180 185 190

Leu Thr Phe Ser Pro Glu Tyr Glu His Ile Phe Asn Asp Ile Ser Gly
195 200 205

Gly Asn His Asn Ser Thr Glu Ser Phe Ile Ala Asp Pro Ala Ile Ser
210 215 220

Leu Ala His Glu Leu Ile His Ala Leu His Gly Leu Tyr Gly Ala Lys
225 230 235 240

Ala Val Thr His Lys Glu Ser Leu Val Ala Glu Arg Gly Pro Leu Met
245 250 255

Ile Ala Glu Lys Pro Ile Arg Leu Glu Glu Phe Leu Thr Phe Gly Gly
260 265 270

Glu Asp Leu Asn Ile Ile Pro Ser Ala Met Lys Glu Lys Ile Tyr Asn
275 280 285

Asp Leu Leu Ala Asn Tyr Glu Lys Ile Ala Thr Arg Leu Arg Glu Val
290 295 300

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Asn Thr Ala Pro Pro Gly Tyr Asp Ile Asn Glu Tyr Lys Asp Tyr Phe
305                 310                 315                 320

Gln Trp Lys Tyr Gly Leu Asp Arg Asn Ala Asp Gly Ser Tyr Thr Val
                325                 330                 335

Asn Arg Asn Lys Phe Asn Glu Ile Tyr Lys Lys Leu Tyr Ser Phe Thr
            340                 345                 350

Glu Ile Asp Leu Ala Asn Lys Phe Lys Val Lys Cys Arg Asn Thr Tyr
        355                 360                 365

Phe Ile Lys Tyr Gly Phe Val Lys Val Pro Asn Leu Leu Asp Asp Asp
    370                 375                 380

Ile Tyr Thr Val Ser Glu Gly Phe Asn Ile Gly Asn Leu Ala Val Asn
385                 390                 395                 400

Asn Arg Gly Gln Asn Ile Asn Leu Asn Pro Lys Ile Ile Asp Ser Ile
                405                 410                 415

Pro Asp Lys Gly Leu Val Glu Lys Ile Ile Lys Phe Cys Lys Ser Ile
            420                 425                 430

Ile Pro Arg Lys Gly Thr Lys Gln Ser Pro Ser Leu Cys Ile Arg Val
        435                 440                 445

Asn Asn Arg Glu Leu Phe Phe Val Ala Ser Glu Ser Ser Tyr Asn Glu
    450                 455                 460

Ser Asp Ile Asn Thr Pro Lys Glu Ile Asp Asp Thr Thr Asn Leu Asn
465                 470                 475                 480

Asn Asn Tyr Arg Asn Asn Leu Asp Glu Val Ile Leu Asp Tyr Asn Ser
                485                 490                 495

Glu Thr Ile Pro Gln Ile Ser Asn Arg Thr Leu Asn Thr Leu Val Gln
            500                 505                 510

Asp Asn Ser Tyr Val Pro Arg Tyr Asp Ser Asn Gly Thr Ser Glu Ile
        515                 520                 525

Glu Glu Tyr Asp Val Val Asp Phe Asn Val Phe Phe Tyr Leu His Ala
    530                 535                 540

Gln Lys Val Pro Glu Gly Glu Thr Asn Ile Ser Leu Thr Ser Ser Ile
545                 550                 555                 560

Asp Thr Ala Leu Leu Glu Glu Ser Lys Val Tyr Thr Phe Phe Ser Ser
                565                 570                 575

Glu Phe Ile Asp Thr Ile Asn Lys Pro Val Asn Ala Ala Leu Phe Ile
            580                 585                 590

Asp Trp Ile Ser Lys Val Ile Arg Asp Phe Thr Thr Glu Ala Thr Gln
        595                 600                 605

Lys Ser Thr Val Asp Lys Ile Ala Asp Ile Ser Leu Ile Val Pro Tyr
    610                 615                 620

Val Gly Leu Ala Leu Asn Ile Val Ile Glu Ala Glu Lys Gly Asn Phe
625                 630                 635                 640

Glu Glu Ala Phe Glu Leu Leu Gly Ala Gly Ile Leu Leu Glu Phe Val
                645                 650                 655

Pro Glu Leu Thr Ile Pro Val Ile Leu Val Phe Thr Ile Lys Ser Tyr
            660                 665                 670

Ile Asp Ser Tyr Glu Asn Lys Asn Lys Ala Ile Lys Ala Ile Asn Asn
        675                 680                 685

Ser Leu Ile Glu Arg Glu Ala Lys Trp Lys Glu Ile Tyr Ser Trp Ile
    690                 695                 700

Val Ser Asn Trp Leu Thr Arg Ile Asn Thr Gln Phe Asn Lys Arg Lys
705                 710                 715                 720

Glu Gln Met Tyr Gln Ala Leu Gln Asn Gln Val Asp Ala Ile Lys Thr
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                725                 730                 735

Ala Ile Glu Tyr Lys Tyr Asn Asn Tyr Thr Ser Asp Glu Lys Asn Arg
            740                 745                 750

Leu Glu Ser Lys Tyr Asn Ile Asn Asn Ile Glu Glu Glu Leu Asn Lys
        755                 760                 765

Lys Val Ser Leu Ala Met Lys Asn Ile Glu Arg Phe Met Thr Glu Ser
    770                 775                 780

Ser Ile Ser Tyr Leu Met Lys Leu Ile Asn Glu Ala Glu Val Gly Lys
785                 790                 795                 800

Leu Lys Glu Tyr Asp Lys His Val Lys Ser Asp Leu Leu Asp Tyr Ile
                805                 810                 815

Leu Tyr His Lys Leu Ile Leu Gly Glu Gln Thr Lys Glu Leu Ile Asp
            820                 825                 830

Leu Val Thr Ser Thr Leu Asn Ser Ser Ile Pro Phe Glu Leu Ser Ser
        835                 840                 845

Tyr Thr Asn Asp Lys Ile Leu Ile Ile Tyr Phe Asn Arg Leu Tyr Lys
    850                 855                 860

Lys Ile Lys Asp Ser Ser Ile Leu Asp Met Arg Tyr Glu Asn Asn Lys
865                 870                 875                 880

Phe Ile Asp Ile Ser Gly Tyr Gly Ser Asn Ile Ser Ile Asn Gly Asn
                885                 890                 895

Val Tyr Ile Tyr Ser Thr Asn Arg Asn Gln Phe Gly Ile Tyr Ser Gly
            900                 905                 910

Arg Leu Ser Glu Val Asn Ile Ala Gln Asn Asn Asp Ile Ile Tyr Asn
        915                 920                 925

Ser Arg Tyr Gln Asn Phe Ser Ile Ser Phe Trp Val Thr Ile Pro Lys
    930                 935                 940

His Tyr Arg Pro Met Asn Arg Asn Arg Glu Tyr Thr Ile Ile Asn Cys
945                 950                 955                 960

Met Gly Asn Asn Asn Ser Gly Trp Lys Ile Ser Leu Arg Thr Ile Arg
                965                 970                 975

Asp Cys Glu Ile Ile Trp Thr Leu Gln Asp Thr Ser Gly Asn Lys Glu
            980                 985                 990

Lys Leu Ile Phe Arg Tyr Glu Glu  Leu Ala Ser Ile Ser  Asp Tyr Ile
        995                 1000                1005

Asn Lys  Trp Ile Phe Val Thr  Ile Thr Asn Asn Arg  Leu Gly Asn
    1010                1015                1020

Ser Arg  Ile Tyr Ile Asn Gly  Asn Leu Ile Val Glu  Lys Ser Ile
    1025                1030                1035

Ser Asn  Leu Gly Asp Ile His  Val Ser Asp Asn Ile  Leu Phe Lys
    1040                1045                1050

Ile Val  Gly Cys Asp Asp Glu  Thr Tyr Val Gly Ile  Arg Tyr Phe
    1055                1060                1065

Lys Val  Phe Asn Thr Glu Leu  Asp Lys Thr Glu Ile  Glu Thr Leu
    1070                1075                1080

Tyr Ser  Asn Glu Pro Asp Pro  Ser Ile Leu Lys Asp  Tyr Trp Gly
    1085                1090                1095

Asn Tyr  Leu Leu Tyr Asn Lys  Lys Tyr Tyr Leu Phe  Asn Leu Leu
    1100                1105                1110

Arg Lys  Asp Lys Tyr Ile Thr  Arg Asn Ser Gly Ile  Leu Asn Ile
    1115                1120                1125

Asn Gln  Gln Arg Gly Val Thr  Gly Gly Ile Ser Val  Phe Leu Asn
    1130                1135                1140
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Tyr Lys  Leu Tyr Glu Gly Val  Glu Val Ile Ile Arg  Lys Asn Ala
    1145                1150                1155

Pro Ile  Asp Ile Ser Asn Thr  Asp Asn Phe Val Arg  Lys Asn Asp
    1160                1165                1170

Leu Ala  Tyr Ile Asn Val Val  Asp His Gly Val Glu  Tyr Arg Leu
    1175                1180                1185

Tyr Ala  Asp Ile Ser Ile Thr  Lys Ser Glu Lys Ile  Ile Lys Leu
    1190                1195                1200

Ile Arg  Thr Ser Asn Pro Asn  Asp Ser Leu Gly Gln  Ile Ile Val
    1205                1210                1215

Met Asp  Ser Ile Gly Asn Asn  Cys Thr Met Asn Phe  Gln Asn Asn
    1220                1225                1230

Asp Gly  Ser Asn Ile Gly Leu  Leu Gly Phe His Ser  Asp Asp Leu
    1235                1240                1245

Val Ala  Ser Ser Trp Tyr Tyr  Asn His Ile Arg Arg  Asn Thr Ser
    1250                1255                1260

Ser Asn  Gly Cys Phe Trp Ser  Phe Ile Ser Lys Glu  His Gly Trp
    1265                1270                1275

Lys Glu
    1280
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<210> SEQ ID NO 13
<211> LENGTH: 3894
<212> TYPE: DNA
<213> ORGANISM: Clostridium botulinum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 13

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atgccagtta atataaaaan ctttaattat aatgacccta ttaataatga tgacattatt     60

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gatcgtattt ggatagtacc agaaaggttt acttatggat ttcaacctga ccaatttaat    180

gccagtacag gagtttttag taaagatgtc tacgaatatt acgatccaac ttatttaaaa    240

accgatgctg aaaaagataa atttttaaaa acaatgatta aattatttaa tagaattaat    300

tcaaaaccat caggacagag attactggat atgatagtag atgctatacc ttatcttgga    360

aatgcatcta caccgcccga caaatttgca gcaaatgttg caaatgtatc tattaataaa    420

aaaattatcc aacctggagc tgaagatcaa ataaaaggtt taatgacaaa tttaataata    480

tttggaccag gaccagttct aagtgataat tttactgata gtatgattat gaatggccat    540

tccccaatat cagaaggatt tggtgcaaga atgatgataa gattttgtcc tagttgttta    600

aatgtattta ataatgttca ggaaaataaa gatacatcta tatttagtag acgcgcgtat    660

tttgcagatc cagctctaac gttaatgcat gaacttatac atgtgttaca tggattatat    720

ggaattaaga taagtaattt accaattact ccaaatacaa aagaattttt catgcaacat    780

agcgatcctg tacaagcaga agaactatat acattcggag gacatgatcc tagtgttata    840

agtccttcta cggatatgaa tatttataat aaagcgttac aaaattttca agatatagct    900

aataggctta atattgtttc aagtgcccaa gggagtggaa ttgatatttc cttatataaa    960

caaatatata aaaataaata tgattttgtt gaagatccta atggaaaata tagtgtagat   1020

aaggataagt ttgataaatt atataaggcc ttaatgtttg gctttactga aactaatcta   1080
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gctggtgaat atggaataaa aactaggtat tcttatttta gtgaatattt gccaccgata 1140

aaaactgaaa aattgttaga caatacaatt tatactcaaa atgaaggctt taacatagct 1200

agtaaaaatc tcaaaacgga atttaatggt cagaataagg cggtaaataa agaggcttat 1260

gaagaaatca gcctagaaca tctcgttata tatagaatag caatgtgcaa gcctgtaatg 1320

tacaaaaata ccggtaaatc tgaacagtgt attattgtta ataatgagga tttatttttc 1380

atagctaata aagatagttt ttcaaaagat ttagctaaag cagaaactat agcatataat 1440

acacaaaata atactataga aaataatttt tctatagatc agttgatttt agataatgat 1500

ttaagcagtg gcatagactt accaaatgaa aacacagaac catttacaaa ttttgacgac 1560

atagatatcc ctgtgtatat taaacaatct gctttaaaaa aaatttttgt ggatggagat 1620

agccttttg aatatttaca tgctcaaaca tttccttcta atatagaaaa tctacaacta 1680

acgaattcat taaatgatgc tttaagaaat aataataaag tctatacttt tttttctaca 1740

aaccttgttg aaaaagctaa tacagttgta ggtgcttcac tttttgtaaa ctgggtaaaa 1800

ggagtaatag atgattttac atctgaatcc acacaaaaaa gtactataga taaagtttca 1860

gatgtatcca taattattcc ctatatagga cctgctttga atgtaggaaa tgaaacagct 1920

aaagaaaatt ttaaaaatgc ttttgaaata ggtggagccg ctatcttaat ggagtttatt 1980

ccagaactta ttgtacctat agttggattt tttacattag aatcatatgt aggaaataaa 2040

gggcatatta ttatgacgat atccaatgct ttaaagaaaa gggatcaaaa atggacagat 2100

atgtatggtt tgatagtatc gcagtggctc tcaacggtta atactcaatt ttatacaata 2160

aaagaaagaa tgtacaatgc tttaaataat caatcacaag caatagaaaa aataatagaa 2220

gatcaatata atagatatag tgaagaagat aaaatgaata ttaacattga ttttaatgat 2280

atagatttta aacttaatca aagtataaat ttagcaataa acaatataga tgattttata 2340

aaccaatgtt ctatatcata tctaatgaat agaatgattc cattagctgt aaaaaagtta 2400

aaagactttg atgataatct taagagagat ttattggagt atatagatac aaatgaacta 2460

tatttacttg atgaagtaaa tattctaaaa tcaaaagtaa atagacacct aaaagacagt 2520

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tatattagta atattagtag taatgctatt ttaagtttaa gttatagagg tgggcgttta 2640

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agtaaattcg ttgtatatga tagtatgttt gataatttta gcattaactt ttgggtaagg 2820

actcctaaat ataataataa tgatatacaa acttatcttc aaaatgagta tacaataatt 2880

agttgtataa aaaatgactc aggatggaaa gtatctatta agggaaatag aataatatgg 2940

acattaatag atgttaatgc aaaatctaaa tcaatatttt tcgaatatag tataaaagat 3000

aatatatcag attatataaa taaatggttt tccataacta ttactaatga tagattaggt 3060

aacgcaaata tttatataaa tggaagtttg aaaaaaagtg aaaaaatttt aaacttagat 3120

agaattaatt ctagtaatga tatagacttc aaattaatta attgtacaga tactactaaa 3180

tttgtttgga ttaaggattt taatattttt ggtagagaat taaatgctac agaagtatct 3240

tcactatatt ggattcaatc atctacaaat actttaaaag atttttgggg gaatccttta 3300

agatacgata cacaatacta tctgtttaat caaggtatgc aaaatatcta tataaagtat 3360

tttagtaaag cttctatggg ggaaactgca ccacgtacaa actttaataa tgcagcaata 3420

aattatcaaa atttatatct tggtttacga tttattataa aaaaagcatc aaattctcgg 3480

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aatataaata atgataatat agtcagagaa ggagattata tatatcttaa tattgataat 3540

atttctgatg aatcttacag agtatatgtt ttggtgaatt ctaaagaaat tcaaactcaa 3600

ttatttttag cacccataaa tgatgatcct acgttctatg atgtactaca aataaaaaaa 3660

tattatgaaa aaacaacata taattgtcag atactttgcg aaaaagatac taaaacattt 3720

gggctgtttg gaattggtaa atttgttaaa gattatggat atgtttggga tacctatgat 3780

aattattttt gcataagtca gtggtatctc agaagaatat ctgaaaatat aaataaatta 3840

aggttgggat gtaattggca attcattccc gtggatgaag gatggacaga ataa 3894

<210> SEQ ID NO 14
<211> LENGTH: 1297
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 14

Met Pro Val Asn Ile Lys Xaa Phe Asn Tyr Asn Asp Pro Ile Asn Asn
1 5 10 15

Asp Asp Ile Ile Met Met Glu Pro Phe Asn Asp Pro Gly Pro Gly Thr
20 25 30

Tyr Tyr Lys Ala Phe Arg Ile Ile Asp Arg Ile Trp Ile Val Pro Glu
35 40 45

Arg Phe Thr Tyr Gly Phe Gln Pro Asp Gln Phe Asn Ala Ser Thr Gly
50 55 60

Val Phe Ser Lys Asp Val Tyr Glu Tyr Tyr Asp Pro Thr Tyr Leu Lys
65 70 75 80

Thr Asp Ala Glu Lys Asp Lys Phe Leu Lys Thr Met Ile Lys Leu Phe
85 90 95

Asn Arg Ile Asn Ser Lys Pro Ser Gly Gln Arg Leu Leu Asp Met Ile
100 105 110

Val Asp Ala Ile Pro Tyr Leu Gly Asn Ala Ser Thr Pro Pro Asp Lys
115 120 125

Phe Ala Ala Asn Val Ala Asn Val Ser Ile Asn Lys Lys Ile Ile Gln
130 135 140

Pro Gly Ala Glu Asp Gln Ile Lys Gly Leu Met Thr Asn Leu Ile Ile
145 150 155 160

Phe Gly Pro Gly Pro Val Leu Ser Asp Asn Phe Thr Asp Ser Met Ile
165 170 175

Met Asn Gly His Ser Pro Ile Ser Glu Gly Phe Gly Ala Arg Met Met
180 185 190

Ile Arg Phe Cys Pro Ser Cys Leu Asn Val Phe Asn Asn Val Gln Glu
195 200 205

Asn Lys Asp Thr Ser Ile Phe Ser Arg Arg Ala Tyr Phe Ala Asp Pro
210 215 220

Ala Leu Thr Leu Met His Glu Leu Ile His Val Leu His Gly Leu Tyr
225 230 235 240

Gly Ile Lys Ile Ser Asn Leu Pro Ile Thr Pro Asn Thr Lys Glu Phe
245 250 255

Phe Met Gln His Ser Asp Pro Val Gln Ala Glu Glu Leu Tyr Thr Phe
260 265 270

Gly Gly His Asp Pro Ser Val Ile Ser Pro Ser Thr Asp Met Asn Ile

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        275                 280                 285

Tyr Asn Lys Ala Leu Gln Asn Phe Gln Asp Ile Ala Asn Arg Leu Asn
    290                 295                 300

Ile Val Ser Ser Ala Gln Gly Ser Gly Ile Asp Ile Ser Leu Tyr Lys
305                 310                 315                 320

Gln Ile Tyr Lys Asn Lys Tyr Asp Phe Val Glu Asp Pro Asn Gly Lys
                325                 330                 335

Tyr Ser Val Asp Lys Asp Lys Phe Asp Lys Leu Tyr Lys Ala Leu Met
            340                 345                 350

Phe Gly Phe Thr Glu Thr Asn Leu Ala Gly Glu Tyr Gly Ile Lys Thr
        355                 360                 365

Arg Tyr Ser Tyr Phe Ser Glu Tyr Leu Pro Pro Ile Lys Thr Glu Lys
    370                 375                 380

Leu Leu Asp Asn Thr Ile Tyr Thr Gln Asn Glu Gly Phe Asn Ile Ala
385                 390                 395                 400

Ser Lys Asn Leu Lys Thr Glu Phe Asn Gly Gln Asn Lys Ala Val Asn
                405                 410                 415

Lys Glu Ala Tyr Glu Glu Ile Ser Leu Glu His Leu Val Ile Tyr Arg
            420                 425                 430

Ile Ala Met Cys Lys Pro Val Met Tyr Lys Asn Thr Gly Lys Ser Glu
        435                 440                 445

Gln Cys Ile Ile Val Asn Asn Glu Asp Leu Phe Phe Ile Ala Asn Lys
    450                 455                 460

Asp Ser Phe Ser Lys Asp Leu Ala Lys Ala Glu Thr Ile Ala Tyr Asn
465                 470                 475                 480

Thr Gln Asn Asn Thr Ile Glu Asn Asn Phe Ser Ile Asp Gln Leu Ile
                485                 490                 495

Leu Asp Asn Asp Leu Ser Ser Gly Ile Asp Leu Pro Asn Glu Asn Thr
            500                 505                 510

Glu Pro Phe Thr Asn Phe Asp Asp Ile Asp Ile Pro Val Tyr Ile Lys
        515                 520                 525

Gln Ser Ala Leu Lys Lys Ile Phe Val Asp Gly Asp Ser Leu Phe Glu
    530                 535                 540

Tyr Leu His Ala Gln Thr Phe Pro Ser Asn Ile Glu Asn Leu Gln Leu
545                 550                 555                 560

Thr Asn Ser Leu Asn Asp Ala Leu Arg Asn Asn Asn Lys Val Tyr Thr
                565                 570                 575

Phe Phe Ser Thr Asn Leu Val Glu Lys Ala Asn Thr Val Val Gly Ala
            580                 585                 590

Ser Leu Phe Val Asn Trp Val Lys Gly Val Ile Asp Asp Phe Thr Ser
        595                 600                 605

Glu Ser Thr Gln Lys Ser Thr Ile Asp Lys Val Ser Asp Val Ser Ile
    610                 615                 620

Ile Ile Pro Tyr Ile Gly Pro Ala Leu Asn Val Gly Asn Glu Thr Ala
625                 630                 635                 640

Lys Glu Asn Phe Lys Asn Ala Phe Glu Ile Gly Gly Ala Ala Ile Leu
                645                 650                 655

Met Glu Phe Ile Pro Glu Leu Ile Val Pro Ile Val Gly Phe Phe Thr
            660                 665                 670

Leu Glu Ser Tyr Val Gly Asn Lys Gly His Ile Ile Met Thr Ile Ser
        675                 680                 685

Asn Ala Leu Lys Lys Arg Asp Gln Lys Trp Thr Asp Met Tyr Gly Leu
    690                 695                 700
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Ile Val Ser Gln Trp Leu Ser Thr Val Asn Thr Gln Phe Tyr Thr Ile
705                 710                 715                 720

Lys Glu Arg Met Tyr Asn Ala Leu Asn Asn Gln Ser Gln Ala Ile Glu
                725                 730                 735

Lys Ile Ile Glu Asp Gln Tyr Asn Arg Tyr Ser Glu Glu Asp Lys Met
            740                 745                 750

Asn Ile Asn Ile Asp Phe Asn Asp Ile Asp Phe Lys Leu Asn Gln Ser
        755                 760                 765

Ile Asn Leu Ala Ile Asn Asn Ile Asp Asp Phe Ile Asn Gln Cys Ser
    770                 775                 780

Ile Ser Tyr Leu Met Asn Arg Met Ile Pro Leu Ala Val Lys Lys Leu
785                 790                 795                 800

Lys Asp Phe Asp Asp Asn Leu Lys Arg Asp Leu Leu Glu Tyr Ile Asp
                805                 810                 815

Thr Asn Glu Leu Tyr Leu Leu Asp Glu Val Asn Ile Leu Lys Ser Lys
            820                 825                 830

Val Asn Arg His Leu Lys Asp Ser Ile Pro Phe Asp Leu Ser Leu Tyr
        835                 840                 845

Thr Lys Asp Thr Ile Leu Ile Gln Val Phe Asn Asn Tyr Ile Ser Asn
    850                 855                 860

Ile Ser Ser Asn Ala Ile Leu Ser Leu Ser Tyr Arg Gly Gly Arg Leu
865                 870                 875                 880

Ile Asp Ser Ser Gly Tyr Gly Ala Thr Met Asn Val Gly Ser Asp Val
                885                 890                 895

Ile Phe Asn Asp Ile Gly Asn Gly Gln Phe Lys Leu Asn Asn Ser Glu
            900                 905                 910

Asn Ser Asn Ile Thr Ala His Gln Ser Lys Phe Val Val Tyr Asp Ser
        915                 920                 925

Met Phe Asp Asn Phe Ser Ile Asn Phe Trp Val Arg Thr Pro Lys Tyr
    930                 935                 940

Asn Asn Asn Asp Ile Gln Thr Tyr Leu Gln Asn Glu Tyr Thr Ile Ile
945                 950                 955                 960

Ser Cys Ile Lys Asn Asp Ser Gly Trp Lys Val Ser Ile Lys Gly Asn
                965                 970                 975

Arg Ile Ile Trp Thr Leu Ile Asp Val Asn Ala Lys Ser Lys Ser Ile
            980                 985                 990

Phe Phe Glu Tyr Ser Ile Lys Asp  Asn Ile Ser Asp Tyr  Ile Asn Lys
        995                 1000                1005

Trp Phe  Ser Ile Thr Ile Thr  Asn Asp Arg Leu Gly  Asn Ala Asn
    1010                1015                1020

Ile Tyr  Ile Asn Gly Ser Leu  Lys Lys Ser Glu Lys  Ile Leu Asn
    1025                1030                1035

Leu Asp  Arg Ile Asn Ser Ser  Asn Asp Ile Asp Phe  Lys Leu Ile
    1040                1045                1050

Asn Cys  Thr Asp Thr Thr Lys  Phe Val Trp Ile Lys  Asp Phe Asn
    1055                1060                1065

Ile Phe  Gly Arg Glu Leu Asn  Ala Thr Glu Val Ser  Ser Leu Tyr
    1070                1075                1080

Trp Ile  Gln Ser Ser Thr Asn  Thr Leu Lys Asp Phe  Trp Gly Asn
    1085                1090                1095

Pro Leu  Arg Tyr Asp Thr Gln  Tyr Tyr Leu Phe Asn  Gln Gly Met
    1100                1105                1110
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Gln Asn  Ile Tyr Ile Lys Tyr  Phe Ser Lys Ala Ser  Met Gly Glu
    1115                1120                1125

Thr Ala  Pro Arg Thr Asn Phe  Asn Asn Ala Ala Ile  Asn Tyr Gln
    1130                1135                1140

Asn Leu  Tyr Leu Gly Leu Arg  Phe Ile Ile Lys Lys  Ala Ser Asn
    1145                1150                1155

Ser Arg  Asn Ile Asn Asn Asp  Asn Ile Val Arg Glu  Gly Asp Tyr
    1160                1165                1170

Ile Tyr  Leu Asn Ile Asp Asn  Ile Ser Asp Glu Ser  Tyr Arg Val
    1175                1180                1185

Tyr Val  Leu Val Asn Ser Lys  Glu Ile Gln Thr Gln  Leu Phe Leu
    1190                1195                1200

Ala Pro  Ile Asn Asp Asp Pro  Thr Phe Tyr Asp Val  Leu Gln Ile
    1205                1210                1215

Lys Lys  Tyr Tyr Glu Lys Thr  Thr Tyr Asn Cys Gln  Ile Leu Cys
    1220                1225                1230

Glu Lys  Asp Thr Lys Thr Phe  Gly Leu Phe Gly Ile  Gly Lys Phe
    1235                1240                1245

Val Lys  Asp Tyr Gly Tyr Val  Trp Asp Thr Tyr Asp  Asn Tyr Phe
    1250                1255                1260

Cys Ile  Ser Gln Trp Tyr Leu  Arg Arg Ile Ser Glu  Asn Ile Asn
    1265                1270                1275

Lys Leu  Arg Leu Gly Cys Asn  Trp Gln Phe Ile Pro  Val Asp Glu
    1280                1285                1290

Gly Trp  Thr Glu
    1295
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<210> SEQ ID NO 15
<211> LENGTH: 4400
<212> TYPE: DNA
<213> ORGANISM: Clostridium tetani

<400> SEQUENCE: 15

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ttaattatga ataatattct taagataaaa agtaaatttt taaaaattta aattttcagt    120

ttacaaaaaa taacctgatt atgttatatg taattgtaaa aaacatataa aaaatcagaa    180

aaatttagga ggtatattat taatggatta aataataatt ttttaattta cttttgatta    240

ataaatatta aatgtttatt ttaattagga gatgatacgt atgccaataa ccataaataa    300

ttttagatat agtgatcctg ttaataatga tacaattatt atgatggagc caccatactg    360

taagggtcta gatatctatt ataaggcttt caaaataaca gatcgtattt ggatagtgcc    420

ggaaaggtat gaatttggga caaaacctga agattttaac ccaccatctt cattaataga    480

aggtgcatct gagtattacg atccaaatta tttaaggact gattctgata aagatagatt    540

tttacaaacc atggtaaaac tgtttaacag aattaaaaac aatgtagcag gtgaagcctt    600

attagataag ataataaatg ccatacctta ccttggaaat tcatattcct tactagacaa    660

gtttgataca aactctaatt cagtatcttt taatttatta gaacaagacc ccagtggagc    720

aactacaaaa tcagcaatgc tgacaaattt aataatattt ggacctgggc ctgttttaaa    780

taaaaatgag gttagaggta ttgtattgag ggtagataat aaaaattact tcccatgtag    840

agatggtttt ggctcaataa tgcaaatggc attttgccca gaatatgtac ctacctttga    900

taatgtaata gaaaatatta cgtcactcac tattggcaaa agcaaatatt ttcaagatcc    960
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agcattacta ttaatgcacg aacttataca tgtactacat ggtttatacg gaatgcaggt 1020

atcaagccat gaaattattc catccaaaca agaaatttat atgcagcata catatccaat 1080

aagtgctgaa gaactattca cttttggcgg acaggatgct aatcttataa gtattgatat 1140

aaaaaacgat ttatatgaaa aaactttaaa tgattataaa gctatagcta acaaacttag 1200

tcaagtcact agctgcaatg atcccaacat tgatattgat agctacaaac aaatatatca 1260

acaaaaatat caattcgata aagatagcaa tggacaatat attgtaaatg aggataaatt 1320

tcagatacta tataatagca taatgtatgg ttttacagag attgaattgg gaaaaaaatt 1380

taatataaaa actagacttt cttattttag tatgaatcat gaccctgtaa aaattccaaa 1440

tttattagat gatacaattt acaatgatac agaaggattt aatatagaaa gcaaagatct 1500

gaaatctgaa tataaaggac aaaatatgag ggtaaataca aatgctttta gaaatgttga 1560

tggatcaggc ctagtttcaa aacttattgg cttatgtaaa aaaattatac caccaacaaa 1620

tataagagaa aatttatata atagaactgc atcattaaca gatttaggag gagaattatg 1680

tataaaaatt aaaaatgaag atttaacttt tatagctgaa aaaaatagct tttcagaaga 1740

accatttcaa gatgaaatag ttagttataa tacaaaaaat aaaccattaa attttaatta 1800

ttcgctagat aaaattattg tagattataa tctacaaagt aaaattacat tacctaatga 1860

taggacaacc ccagttacaa aaggaattcc atatgctcca gaatataaaa gtaatgctgc 1920

aagtacaata gaaatacata atattgatga caatacaata tatcaatatt tgtatgctca 1980

aaaatctcct acaactctac aaagaataac tatgactaat tctgttgatg acgcattaat 2040

aaattccacc aaaatatatt catattttcc atctgtaatc agtaaagtta accaaggtgc 2100

acaaggaatt ttattcttac agtgggtgag agatataatt gatgatttta ccaatgaatc 2160

ttcacaaaaa actactattg ataaaatttc agatgtatcc actattgttc cttatatagg 2220

acccgcatta aacattgtaa aacaaggcta tgagggaaac tttataggcg ctttagaaac 2280

taccggagtg gttttattat tagaatatat tccagaaatt actttaccag taattgcagc 2340

tttatctata gcagaaagta gcacacaaaa agaaaagata ataaaaacaa tagataactt 2400

tttagaaaaa agatatgaaa aatggattga agtatataaa ctagtaaaag caaaatggtt 2460

aggcacagtt aatacgcaat tccaaaaaag aagttatcaa atgtatagat ctttagaata 2520

tcaagtagat gcaataaaaa aaataataga ctatgaatat aaaatatatt caggacctga 2580

taaggaacaa attgccgacg aaattaataa tctgaaaaac aaacttgaag aaaaggctaa 2640

taaagcaatg ataaacataa atatatttat gagggaaagt tctagatcat ttttagttaa 2700

tcaaatgatt aacgaagcta aaaagcagtt attagagttt gatactcaaa gcaaaaatat 2760

tttaatgcag tatataaaag caaattctaa atttataggt ataactgaac taaaaaaatt 2820

agaatcaaaa ataaacaaag ttttttcaac accaattcca ttttcttatt ctaaaaatct 2880

ggattgttgg gttgataatg aagaagatat agatgttata ttaaaaaaga gtacaatttt 2940

aaatttagat attaataatg atattatatc agatatatct gggtttaatt catctgtaat 3000

aacatatcca gatgctcaat tggtgcccgg aataaatggc aaagcaatac atttagtaaa 3060

caatgaatct tctgaagtta tagtgcataa agctatggat attgaatata atgatatgtt 3120

taataatttt accgttagct tttggttgag ggttcctaaa gtatctgcta gtcatttaga 3180

acaatatggc acaaatgagt attcaataat tagctctatg aaaaaacata gtctatcaat 3240

aggatctggt tggagtgtat cacttaaagg taataactta atatggactt taaaagattc 3300

cgcgggagaa gttagacaaa taacttttag ggatttacct gataaattta atgcttattt 3360

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agcaaataaa tgggttttta taactattac taatgataga ttatcttctg ctaatttgta 3420

tataaatgga gtacttatgg gaagtgcaga aattactggt ttaggagcta ttagagagga 3480

taataatata acattaaaac tagatagatg taataataat aatcaatacg tttctattga 3540

taaatttagg atattttgca aagcattaaa tccaaaagag attgaaaaat tatacacaag 3600

ttatttatct ataacctttt taagagactt ctggggaaac cctttacgat atgatacaga 3660

atattattta ataccagtag cttctagttc taaagatgtt caattgaaaa atataacaga 3720

ttatatgtat ttgacaaatg cgccatcgta tactaacgga aaattgaata tatattatag 3780

aaggttatat aatggactaa aatttattat aaaaagatat acacctaata atgaaataga 3840

ttcttttgtt aaatcaggtg attttattaa attatatgta tcatataaca ataatgagca 3900

cattgtaggt tatccgaaag atggaaatgc ctttaataat cttgatagaa ttctaagagt 3960

aggttataat gccccaggta tccctcttta taaaaaaatg gaagcagtaa aattgcgtga 4020

tttaaaaacc tattctgtac aacttaaatt atatgatgat aaaaatgcat ctttaggact 4080

agtaggtacc cataatggtc aaataggcaa cgatccaaat agggatatat taattgcaag 4140

caactggtac tttaatcatt taaaagataa aattttagga tgtgattggt actttgtacc 4200

tacagatgaa ggatggacaa atgattaaac agattgatat gttcatgatt actctatata 4260

aaaaattaaa taatataaca atctagctat attatttttg attattttct taatatatac 4320

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ttttgacaca gcctctactt 4400

<210> SEQ ID NO 16
<211> LENGTH: 1447
<212> TYPE: PRT
<213> ORGANISM: Clostridium tetani

<400> SEQUENCE: 16

Ser Ile Lys Lys Ile Arg Thr Tyr Ser Ile Asn Tyr Ile Val Phe Ile
1 5 10 15

Ile Leu Ile Ile Phe Leu Arg Lys Val Asn Phe Lys Phe Lys Phe Ser
20 25 30

Val Tyr Lys Lys Pro Asp Tyr Val Ile Cys Asn Cys Lys Lys His Ile
35 40 45

Lys Asn Gln Lys Asn Leu Gly Gly Ile Leu Leu Met Asp Ile Ile Ile
50 55 60

Phe Phe Thr Phe Asp Ile Leu Asn Val Tyr Phe Asn Glu Met Ile Arg
65 70 75 80

Met Pro Ile Thr Ile Asn Asn Phe Arg Tyr Ser Asp Pro Val Asn Asn
85 90 95

Asp Thr Ile Ile Met Met Glu Pro Pro Tyr Cys Lys Gly Leu Asp Ile
100 105 110

Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Val Pro Glu
115 120 125

Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser
130 135 140

Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr
145 150 155 160

Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn
165 170 175

Arg Ile Lys Asn Asn Val Ala Gly Glu Ala Leu Leu Asp Lys Ile Ile

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            180                 185                 190

Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Ser Leu Leu Asp Lys Phe
        195                 200                 205

Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro
    210                 215                 220

Ser Gly Ala Thr Thr Lys Ser Ala Met Leu Thr Asn Leu Ile Ile Phe
225                 230                 235                 240

Gly Pro Gly Pro Val Leu Asn Lys Asn Glu Val Arg Gly Ile Val Leu
                245                 250                 255

Arg Val Asp Asn Lys Asn Tyr Phe Pro Cys Arg Asp Gly Phe Gly Ser
            260                 265                 270

Ile Met Gln Met Ala Phe Cys Pro Glu Tyr Val Pro Thr Phe Asp Asn
        275                 280                 285

Val Ile Glu Asn Ile Thr Ser Leu Thr Ile Gly Lys Ser Lys Tyr Phe
    290                 295                 300

Gln Asp Pro Ala Leu Leu Leu Met His Glu Leu Ile His Val Leu His
305                 310                 315                 320

Gly Leu Tyr Gly Met Gln Val Ser Ser His Glu Ile Ile Pro Ser Lys
                325                 330                 335

Gln Glu Ile Tyr Met Gln His Thr Tyr Pro Ile Ser Ala Glu Glu Leu
            340                 345                 350

Phe Thr Phe Gly Gly Gln Asp Ala Asn Leu Ile Ser Ile Asp Ile Lys
        355                 360                 365

Asn Asp Leu Tyr Glu Lys Thr Leu Asn Asp Tyr Lys Ala Ile Ala Asn
    370                 375                 380

Lys Leu Ser Gln Val Thr Ser Cys Asn Asp Pro Asn Ile Asp Ile Asp
385                 390                 395                 400

Ser Tyr Lys Gln Ile Tyr Gln Gln Lys Tyr Gln Phe Asp Lys Asp Ser
                405                 410                 415

Asn Gly Gln Tyr Ile Val Asn Glu Asp Lys Phe Gln Ile Leu Tyr Asn
            420                 425                 430

Ser Ile Met Tyr Gly Phe Thr Glu Ile Glu Leu Gly Lys Lys Phe Asn
        435                 440                 445

Ile Lys Thr Arg Leu Ser Tyr Phe Ser Met Asn His Asp Pro Val Lys
    450                 455                 460

Ile Pro Asn Leu Leu Asp Asp Thr Ile Tyr Asn Asp Thr Glu Gly Phe
465                 470                 475                 480

Asn Ile Glu Ser Lys Asp Leu Lys Ser Glu Tyr Lys Gly Gln Asn Met
                485                 490                 495

Arg Val Asn Thr Asn Ala Phe Arg Asn Val Asp Gly Ser Gly Leu Val
            500                 505                 510

Ser Lys Leu Ile Gly Leu Cys Lys Lys Ile Ile Pro Pro Thr Asn Ile
        515                 520                 525

Arg Glu Asn Leu Tyr Asn Arg Thr Ala Ser Leu Thr Asp Leu Gly Gly
    530                 535                 540

Glu Leu Cys Ile Lys Ile Lys Asn Glu Asp Leu Thr Phe Ile Ala Glu
545                 550                 555                 560

Lys Asn Ser Phe Ser Glu Glu Pro Phe Gln Asp Glu Ile Val Ser Tyr
                565                 570                 575

Asn Thr Lys Asn Lys Pro Leu Asn Phe Asn Tyr Ser Leu Asp Lys Ile
            580                 585                 590

Ile Val Asp Tyr Asn Leu Gln Ser Lys Ile Thr Leu Pro Asn Asp Arg
        595                 600                 605
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Thr Thr Pro Val Thr Lys Gly Ile Pro Tyr Ala Pro Glu Tyr Lys Ser
    610                 615                 620

Asn Ala Ala Ser Thr Ile Glu Ile His Asn Ile Asp Asp Asn Thr Ile
625                 630                 635                 640

Tyr Gln Tyr Leu Tyr Ala Gln Lys Ser Pro Thr Thr Leu Gln Arg Ile
                645                 650                 655

Thr Met Thr Asn Ser Val Asp Asp Ala Leu Ile Asn Ser Thr Lys Ile
            660                 665                 670

Tyr Ser Tyr Phe Pro Ser Val Ile Ser Lys Val Asn Gln Gly Ala Gln
        675                 680                 685

Gly Ile Leu Phe Leu Gln Trp Val Arg Asp Ile Ile Asp Asp Phe Thr
    690                 695                 700

Asn Glu Ser Ser Gln Lys Thr Thr Ile Asp Lys Ile Ser Asp Val Ser
705                 710                 715                 720

Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn Ile Val Lys Gln Gly
                725                 730                 735

Tyr Glu Gly Asn Phe Ile Gly Ala Leu Glu Thr Thr Gly Val Val Leu
            740                 745                 750

Leu Leu Glu Tyr Ile Pro Glu Ile Thr Leu Pro Val Ile Ala Ala Leu
        755                 760                 765

Ser Ile Ala Glu Ser Ser Thr Gln Lys Glu Lys Ile Ile Lys Thr Ile
    770                 775                 780

Asp Asn Phe Leu Glu Lys Arg Tyr Glu Lys Trp Ile Glu Val Tyr Lys
785                 790                 795                 800

Leu Val Lys Ala Lys Trp Leu Gly Thr Val Asn Thr Gln Phe Gln Lys
                805                 810                 815

Arg Ser Tyr Gln Met Tyr Arg Ser Leu Glu Tyr Gln Val Asp Ala Ile
            820                 825                 830

Lys Lys Ile Ile Asp Tyr Glu Tyr Lys Ile Tyr Ser Gly Pro Asp Lys
        835                 840                 845

Glu Gln Ile Ala Asp Glu Ile Asn Asn Leu Lys Asn Lys Leu Glu Glu
    850                 855                 860

Lys Ala Asn Lys Ala Met Ile Asn Ile Asn Ile Phe Met Arg Glu Ser
865                 870                 875                 880

Ser Arg Ser Phe Leu Val Asn Gln Met Ile Asn Glu Ala Lys Lys Gln
                885                 890                 895

Leu Leu Glu Phe Asp Thr Gln Ser Lys Asn Ile Leu Met Gln Tyr Ile
            900                 905                 910

Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Glu
        915                 920                 925

Ser Lys Ile Asn Lys Val Phe Ser Thr Pro Ile Pro Phe Ser Tyr Ser
    930                 935                 940

Lys Asn Leu Asp Cys Trp Val Asp Asn Glu Glu Asp Ile Asp Val Ile
945                 950                 955                 960

Leu Lys Lys Ser Thr Ile Leu Asn Leu Asp Ile Asn Asn Asp Ile Ile
                965                 970                 975

Ser Asp Ile Ser Gly Phe Asn Ser Ser Val Ile Thr Tyr Pro Asp Ala
            980                 985                 990

Gln Leu Val Pro Gly Ile Asn Gly  Lys Ala Ile His Leu  Val Asn Asn
        995                 1000                1005

Glu Ser  Ser Glu Val Ile Val  His Lys Ala Met Asp  Ile Glu Tyr
    1010                1015                1020
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Asn Asp  Met Phe Asn Asn Phe  Thr Val Ser Phe Trp  Leu Arg Val
    1025                 1030                 1035

Pro Lys  Val Ser Ala Ser His  Leu Glu Gln Tyr Gly  Thr Asn Glu
    1040                 1045                 1050

Tyr Ser  Ile Ile Ser Ser Met  Lys Lys His Ser Leu  Ser Ile Gly
    1055                 1060                 1065

Ser Gly  Trp Ser Val Ser Leu  Lys Gly Asn Asn Leu  Ile Trp Thr
    1070                 1075                 1080

Leu Lys  Asp Ser Ala Gly Glu  Val Arg Gln Ile Thr  Phe Arg Asp
    1085                 1090                 1095

Leu Pro  Asp Lys Phe Asn Ala  Tyr Leu Ala Asn Lys  Trp Val Phe
    1100                 1105                 1110

Ile Thr  Ile Thr Asn Asp Arg  Leu Ser Ser Ala Asn  Leu Tyr Ile
    1115                 1120                 1125

Asn Gly  Val Leu Met Gly Ser  Ala Glu Ile Thr Gly  Leu Gly Ala
    1130                 1135                 1140

Ile Arg  Glu Asp Asn Asn Ile  Thr Leu Lys Leu Asp  Arg Cys Asn
    1145                 1150                 1155

Asn Asn  Asn Gln Tyr Val Ser  Ile Asp Lys Phe Arg  Ile Phe Cys
    1160                 1165                 1170

Lys Ala  Leu Asn Pro Lys Glu  Ile Glu Lys Leu Tyr  Thr Ser Tyr
    1175                 1180                 1185

Leu Ser  Ile Thr Phe Leu Arg  Asp Phe Trp Gly Asn  Pro Leu Arg
    1190                 1195                 1200

Tyr Asp  Thr Glu Tyr Tyr Leu  Ile Pro Val Ala Ser  Ser Ser Lys
    1205                 1210                 1215

Asp Val  Gln Leu Lys Asn Ile  Thr Asp Tyr Met Tyr  Leu Thr Asn
    1220                 1225                 1230

Ala Pro  Ser Tyr Thr Asn Gly  Lys Leu Asn Ile Tyr  Tyr Arg Arg
    1235                 1240                 1245

Leu Tyr  Asn Gly Leu Lys Phe  Ile Ile Lys Arg Tyr  Thr Pro Asn
    1250                 1255                 1260

Asn Glu  Ile Asp Ser Phe Val  Lys Ser Gly Asp Phe  Ile Lys Leu
    1265                 1270                 1275

Tyr Val  Ser Tyr Asn Asn Asn  Glu His Ile Val Gly  Tyr Pro Lys
    1280                 1285                 1290

Asp Gly  Asn Ala Phe Asn Asn  Leu Asp Arg Ile Leu  Arg Val Gly
    1295                 1300                 1305

Tyr Asn  Ala Pro Gly Ile Pro  Leu Tyr Lys Lys Met  Glu Ala Val
    1310                 1315                 1320

Lys Leu  Arg Asp Leu Lys Thr  Tyr Ser Val Gln Leu  Lys Leu Tyr
    1325                 1330                 1335

Asp Asp  Lys Asn Ala Ser Leu  Gly Leu Val Gly Thr  His Asn Gly
    1340                 1345                 1350

Gln Ile  Gly Asn Asp Pro Asn  Arg Asp Ile Leu Ile  Ala Ser Asn
    1355                 1360                 1365

Trp Tyr  Phe Asn His Leu Lys  Asp Lys Ile Leu Gly  Cys Asp Trp
    1370                 1375                 1380

Tyr Phe  Val Pro Thr Asp Glu  Gly Trp Thr Asn Asp  Thr Asp Tyr
    1385                 1390                 1395

Val His  Asp Tyr Ser Ile Lys  Ile Lys Tyr Asn Asn  Leu Ala Ile
    1400                 1405                 1410

Leu Phe  Leu Ile Ile Phe Leu  Ile Tyr Thr Asn Lys  Ile Ile Lys
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1415 1420 1425

Ile Glu Pro Ile Leu Asn Tyr Arg Ala Val Ser Lys Asp Phe Asp
1430 1435 1440

Thr Ala Ser Thr
1445

<210> SEQ ID NO 17
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 17

Lys Leu Leu Cys Val Arg Gly Ile Ile Thr Ser Lys Thr Lys Ser Leu
1 5 10 15

Asp Lys Gly Tyr Asn Lys Ala Leu Asn Asp Leu Cys Ile Lys Val
20 25 30

<210> SEQ ID NO 18
<211> LENGTH: 16
<212> TYPE: PRT
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<400> SEQUENCE: 18

Ile Gln Met Cys Lys Ser Val Lys Ala Pro Gly Ile Cys Ile Asp Val
1 5 10 15

<210> SEQ ID NO 19
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 19

Thr Lys Phe Cys His Lys Ala Ile Asp Gly Arg Ser Leu Tyr Asn Lys
1 5 10 15

Thr Leu Asp Cys Arg Glu Leu Leu Val
20 25

<210> SEQ ID NO 20
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 20

Thr Lys Val Cys Leu Arg Leu Thr Lys Asn Ser Arg Asp Asp Ser Thr
1 5 10 15

Cys Ile Lys Val
20

<210> SEQ ID NO 21
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 21

Ile Arg Phe Cys Lys Asn Ile Val Ser Val Lys Gly Ile Arg Lys Ser
1 5 10 15

Ile Cys Ile Glu Ile
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<210> SEQ ID NO 22
<211> LENGTH: 23
<212> TYPE: PRT

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<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 22

Val Lys Phe Cys Lys Ser Val Ile Pro Arg Lys Gly Thr Lys Ala Pro
1 5 10 15

Pro Arg Leu Cys Ile Arg Val
20

<210> SEQ ID NO 23
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 23

Ile Ala Met Cys Lys Pro Val Met Tyr Lys Asn Thr Gly Lys Ser Glu
1 5 10 15

Gln Cys Ile Ile Val
20

<210> SEQ ID NO 24
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 24

Ile Gly Leu Cys Lys Lys Ile Ile Pro Pro Thr Asn Ile Arg Glu Asn
1 5 10 15

Leu Tyr Asn Arg Thr Ala Ser Leu Thr Asp Leu Gly Gly Glu Leu Cys
20 25 30

Ile Lys Ile
35

<210> SEQ ID NO 25
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 25

Ala Leu Ala Pro Tyr Ile Pro
1 5

<210> SEQ ID NO 26
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 26

Arg Phe Met Asp Tyr Trp Glu Gly Leu
1 5

<210> SEQ ID NO 27
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2)..(4)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 27

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Phe Xaa Xaa Xaa Leu Trp Xaa Xaa Leu
1 5

<210> SEQ ID NO 28
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 28

Glu Leu Glu Ser Pro Pro Pro Pro Tyr Ser Arg Tyr Pro Met
1 5 10

<210> SEQ ID NO 29
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 29

Lys Val Gly Phe Phe Lys Arg
1 5

<210> SEQ ID NO 30
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 30

Leu Leu Val Arg Gly Arg Thr Leu Val Val
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum

<400> SEQUENCE: 31

Asp Arg His Asp Ser Gly Leu Asp Ser Met
1 5 10

<210> SEQ ID NO 32
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
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<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
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<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 32

Pro Xaa Ala Xaa Val Xaa Pro
1 5

<210> SEQ ID NO 33
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (5)..(6)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 33

Pro Pro Xaa Tyr Xaa Xaa Met
1 5

<210> SEQ ID NO 34
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Clostridium botulinum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
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<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 34

Pro Pro Xaa Tyr Xaa
1 5

The invention claimed is:

1. A modified neurotoxin polypeptide exhibiting a reduced duration of muscle paralysis in a subject, wherein the neurotoxin polypeptide comprises at least one degradation signal in a light chain of the neurotoxin polypeptide, wherein the degradation signal in the light chain of the neurotoxin polypeptide is selected from:

a) at least one internally or terminally introduced PEST motif;

b) at least one internally or terminally introduced E3 ligase recognition motif;

c) an N-terminal oligo-lysine residue;

d) an N-terminally linked ubiquitin;

e) a substitution of the N-terminal praline with a basic amino acid;

f) substitutions of surface displayed amino acid residues with lysine; and

g) a substitution of the N-terminal praline with a basic amino add in combination with substitutions of surface displayed amino add residues with lysine.

2. The modified neurotoxin polypeptide of claim **1**, wherein the duration of the muscle paralysis in a subject persists less than 4, 3 or 2 weeks.

3. The modified neurotoxin polypeptide of claim **1**, wherein the light chain of the neurotoxin polypeptide exhibiting a reduced duration of muscle paralysis in a subject is a modified form of a neurotoxin light chain having an amino acid sequence set forth as SEQ ID NO; 2, 4, 6, 8, 10, 12, 14, or 16.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,193,771 B2
APPLICATION NO. : 14/056247
DATED : November 24, 2015
INVENTOR(S) : Fred Hofmann et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page, Other Publications: "WO2006/007185 1/2006"
should be
--WO2005/007185 1/2005--.

Title Page, Line 3, Other Publications: "Microbiology. vol. 162"
should be
--Microbiology, vol. 152--.

Title Page, Lines 18-19, Other Publications: "International Search Report for PCT/EP2010/05938"
should be
--International Search Report for PCT/EP2010/059398--.

Title Page, Line 23, Other Publications: "Eur. J. Biochem., vol.188 p. 399-45"
should be
--Eur. J. Biochem., vol.188 p. 339-45--.

Title Page, Line 29, Other Publications: "Science, vol. 234, p. 364-366"
should be
--Science, vol. 234, p. 364-368--.

IN THE CLAIMS

Column 100, Line 30: "praline" should be --proline--.

Column 100, Line 32: "add" should be --acid--.

Signed and Sealed this
Fifth Day of January, 2016

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,193,771 B2
APPLICATION NO. : 14/056247
DATED : November 24, 2015
INVENTOR(S) : Hofmann et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

Column 99, Line 41 Claim 1: “praline” should read --proline--.

Signed and Sealed this
Second Day of January, 2018

Joseph Matal

Joseph Matal
Performing the Functions and Duties of the
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office